International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH
Available online at:http://www.pharmaresearchlibrary.com/ijcps

Analytical Method Development and Validation of Doravirine in Pharmaceutical Dosage Form by RP-HPLC

V. Hari Baskar*, SK. Karishma

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur (V&P), Muthukur (M), SPSR Nellore – 524346

ABSTRACT

A new method was established for estimation of Doravirine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Doravirine by using Agilent column (4.6×150mm) 5µ, flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer: meoH (25:75% v/v), detection wavelength was 270 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.182mins. The % purity of Doravirine was found to be 98.56%.The system suitability parameters for Doravirine such as theoretical plates and tailing factor were found to be 4343.2, 1.6. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)).

Keywords: Agilent column, Doravirine, RP-HPLC

ARTICLE HISTORY: Received 20 February 2020, **Accepted** 29 March 2020, **Available Online** 27 April 2020

Citation: V. Hari Baskar. Analytical Method Development and Validation of Doravirine in Pharmaceutical Dosage Form by RP-HPLC.*International Journal of Chemistry and Pharmaceutical Sciences,* 8(4), 2020: 86-90.

CONTENTS:

Corresponding author
Dr. V. Hari Baskar
Professor and Head
Department of Pharmaceutical Analysis,
Ratnam Institute of Pharmacy, Pidathapolur (V&P),
Muthukur (M), SPSR Nellore – 524346

JOURNAL QR-CODE

1. Introduction

Doravirine is a pyridinone non-nucleoside reverse transcriptase inhibitor of HIV-1. As reverse transcriptase is the principal virally encoded enzyme with which retroviruses like HIV convert their RNA genomes into DNA for the purposes of proliferation within the host genome of infected cells, doravirine subsequently functions by inhibiting HIV-1 replication by the non-competitive inhibition of HIV-1 reverse transcriptase (RT). Doravirine does not however, inhibit the human cellular DNA polymerases α, ß, and mitochondrial DNA polymerase γ. Literature review reveals that there is no analytical method reported for the analysis of Doravirine by estimation by RP-

HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the estimation of Doravirine in pharmaceutical dosage form.

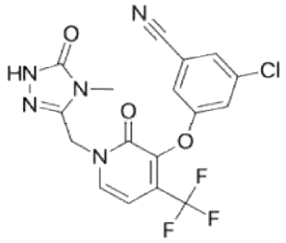

Fig 1: Chemical Structure of Doravirine

2. Materials and Methods

Chemicals and Standards used:

Table 1: List of Chemicals and Reagents

S. No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	Tancodep-2	Torrent pharmaceuticals	Tablet form
8.	Doravirine	In – House	In-House

Instruments used:

Table 2: List of Instruments

S. No	Instrument name	Model number	Mnf. Name
1	HPLC-auto sampler – UV detector	Separation module2695, UV. detector 2487 Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+ U.V win soft ware	Lab India
3	Digital weighing balance	ER 200A	Ascoset
4	pH meter	AD 102U	ADWA
5	Sonicator	SE60US	Enertech

Method development

Selection of wavelength:

10 mg of Doravirine was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Doravirine. The overlay spectrum of Doravirine was obtained and the isobestic point of Doravirine showed absorbance's maxima at 270 nm.

Preparation of mobile phase:

Mix a mixture of 250 ml Phosphate buffer (25%) and 750 ml of Methanol (75%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of the individual Doravirine standard preparation:

10 mg of Doravirine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Sample solution preparation:

10 mg of Doravirine tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

10 mg Doravirine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Optimized Chromatographic Conditions:

Column : Agilent (5µm, 4.6x150mm)
Column temperature : Ambient
Wavelength : 270 nm
Mobile phase ratio (25:75% v/v) : Phosphate buffer: MeoH
Flow rate : 1.0 ml/min
Auto sampler temperature : Ambient
Injection volume : 10µl
Run time : 10.0 minutes

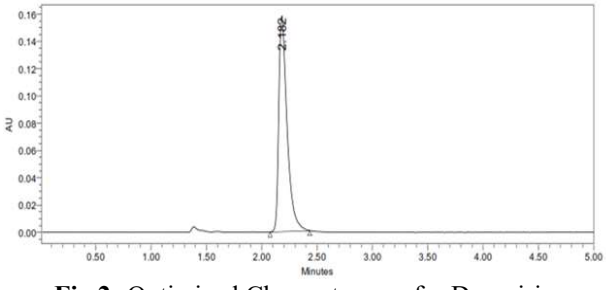

Fig 2: Optimized Chromatogram for Doravirine

System suitability:

- ✓ Tailing factor for the peaks due to Doravirine in standard solution should not be more than 1.5.

- ✓ Theoretical plates for the Doravirine peaks in standard solution should not be less than 2000.

Method Validation

Method validation was carried on according to ICH guidelines Q_2 (R_1). The validation parameters such as specificity, linearity, accuracy, precision, quantification limits and robustness.

3. Results and Discussion

Assay calculation for Doravirine:

The assay study was performed for the Doravirine. Each three injections of sample and standard were injected into chromatographic system. The retention time of Doravirine was found to be 2.142 mins. The system suitability parameters for Doravirine such as theoretical plates and tailing factor were found to be 4343.4, 1.6.The % purity Doravirine in pharmaceutical dosage form was found to be 99.56%.

Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The specificity test was performed for Doravirine. It was found that there was no interference of impurities in retention time of analytical peak.

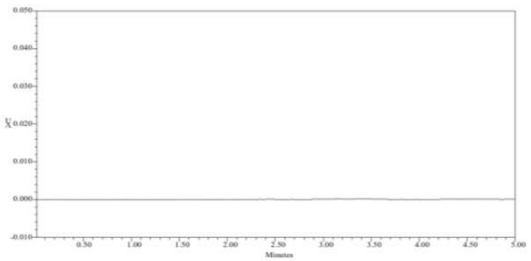

Fig 3: Chromatogram showing blank

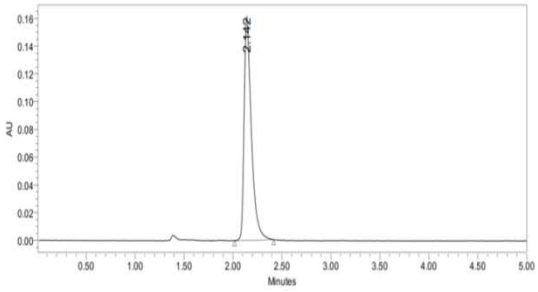

Fig 4: Chromatogram for Standard

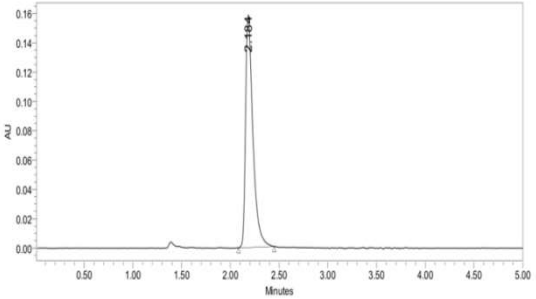

Fig 5: Chromatogram for Sample

Linearity:

The linearity study was performed for the concentration of 20-100 ppm Doravirine. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The linearity study was performed for concentration range of 20µg-100µg Doravirine and the correlation coefficient was found to be 0.999 (NLT 0.999).

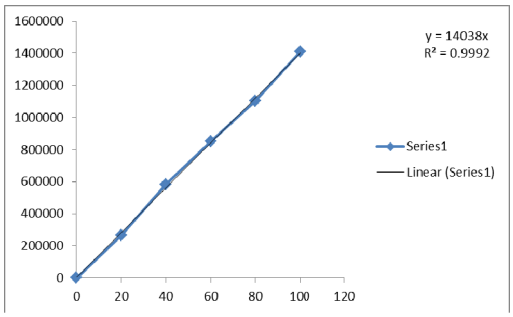

Fig 6: Calibration graph for Doravirine

Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for Doravirine. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The accuracy study was performed for % recovery of Doravirine. The % recovery was found to be 98.96% (NLT 98% and NMT 102%).

Precision

- ❖ Repeatability
- ❖ Intermediate Precision

Repeatability: The precision study was performed for five injections of Doravirine. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD. The Method precision study was performed for the %RSD of Doravirine was found to be 0.3 (NMT 2).

Intermediate precision/Ruggedness:

The intermediate precision study was performed for five injections of Doravirine. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD. The intermediate precision was performed for %RSD of Doravirine was found to be 0.8 (NMT 2).

LOD & LOQ:

- The LOD was performed for Doravirine was found to be 0.7.
- The LOQ was performed for Doravirine was found to be 0.12.

Robustness:

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Doravirine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

Variation in Flow rate: The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change

in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

Variation in Mobile Phase: On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Table 3: Linearity Results

S. No	Linearity Level	Concentration	Area
1	I	20 ppm	265957
2	II	40 ppm	583632
3	III	60 ppm	850130
4	IV	80 ppm	1103642
5	V	100 ppm	1408820
Correlation Coefficient			0.991

Table 4: Accuracy Results

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1143519	5	4.86	98.81%	98.96%
100%	2938342	10	9.88	99.08%	
150%	4452758	15	15.0	100.0%	

Table 5: Repeatability Results

	Peak Name	RT	Area	Height
1	Doravirine	2.185	824170	158772
2	Doravirine	2.191	826053	157336
3	Doravirine	2.204	823442	156124
4	Doravirine	2.207	818967	155674
5	Doravirine	2.210	823476	156033
Mean			823221.9	
Std. Dev.			2604.2	
% RSD			0.3	

Table 6: Intermediate precision results

	Peak Name	RT	Area	Height
1	Doravirine	2.180	830760	160374
2	Doravirine	2.184	832532	160030
3	Doravirine	2.185	823385	159662
4	Doravirine	2.188	840724	161107
5	Doravirine	2.188	829385	160286
Mean			831357.4	
Std. Dev.			6263.2	
% RSD			0.8	

Table 7: Showing system suitability results for Doravirine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	4517	1.7
2	1.0	4343	1.6
3	1.2	4209	1.6

Table 8: Showing system suitability results for Doravirine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	4623	1.6
2	*Actual	4543	1.6
3	5 % more	4864	1.6

4. Conclusion

A new method was established for estimation of Doravirine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Doravirine by using Agilent column (4.6×150mm) 5µ, flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer: meoH (25:75% v/v), detection wavelength was 270 nm. The retention times were found to be 2.182 mins. The % purity of Doravirine was found to be 98.56%.The system suitability parameters for Doravirine such as theoretical plates and tailing factor were found to be 4343.2, 1.6. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Doravirine was found in concentration range of 20µg-100µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 98.96%, %RSD for repeatability was 0.3, % RSD for intermediate precision was 0.8. The precision study was precision, robustness and repeatability. LOD value was 0.7 and LOQ value was 0.12. Hence the suggested RP-HPLC method can be used for routine analysis of Doravirine in API and Pharmaceutical dosage form.

5. References

[1] Becket And Stenlake, Practical Pharmaceutical Chemistry, Part 24th Edition Cbs Publications And Distributors, 2005, 157-168.

[2] P.D. Sethi, Hplc Quantitative Analysis of Pharmaceutical Formulations Cbs Publications And Distributors, 1st Edition, 2001, 69-70.

[3] B.K Sharma, Instrumental Method Of Chemical Analysis, 23rd Edition, Goal Publishers 2004.

[4] Practical Hplc Method Development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, Second Edition, 1, 420-430,686-704.

[5] Validating Chromatographic Methods, David M.Bliesner. 1-4.

[6] International Conference On Harmonization: Ich Q 2 (R1) Validation Of Analytical Procedures: Text And Methodology 1995.

[7] Indian Pharmacopeia 2007 Vol –I Pg.No-715.

[8] British Pharmacopeia 2007 Vol-I Pg.No-136.

[9] Dhara S. Bhavsar,, RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form 2012 Jul-Dec; 3(2): 73–78.

[10] Anandakumar Karunakaran, A Validated RP - HPLC Method for Simulataneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Pure and in Tablet Dosage Form Pelagia Research Library Der Pharmacia Sinica, 2010, 1 (2): 52-60

[11] Anjaneyulu. N et al, Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine and Tenofovir Disproxil Fumerate in Combined Dosage Form. Asian Journal of Biomedical and Pharmaceutical Sciences; 3(23) 2013, 7-11.

[12] Chinnalalaiah Runja et al, A Validated Stability Indicating RP-HPLC Method for the Determination of Emtricitabine, Tenofovir Disoproxil Fumarate, Elvitegravir and Cobicistat in Pharmaceutical Dosage Form Journal of Chromatographic Science, 2016, Vol. 54, No. 5, 759–764.