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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DORAVIRINEIN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

T. Hanuman[1], Dr. T. Sivakkumar[2], Dr. S. Sridhar[1].

[1] Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Maissammaguda, Secunderabad - 500100

[2]Department of Pharmaceutical Chemistry, Annamalai University, Chidambaram, Tamilnadu.

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Abstract:

A simple, Précise, Accurate method was developed for the estimation of Doravirineby RP-HPLC technique. Chromatographic conditions used are stationary phase Agilent C_{18} (250mm X 4.6mm5µ), Mobile phase0.01N KH_2PO_4: Methanol in the ratio of 50:50 and flow rate was maintained at 1.0ml/min, detection wave length was 210nm, column temperature was set to 30°C and diluent was mobile phase conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between25% to150 % levels, R^2 value was found to be as 0.999.LOD and LOQ are 0.20µg/ml and 0.61µg/ml respectively. By using above method assay of marketed formulation was carried out 99.98% was present. Degradation studies of Doravirine were done, in all conditions purity threshold was more than purity angle and within the acceptable range.This method can be used for routine analysis of Doravirine.

Key words: *HPLC Doravirine, Method development. ICH Guidelines.*

Corresponding author:

T. Hanuman,

Department of Pharmaceutical Analysis,
Malla Reddy College of Pharmacy,
Maissammaguda, Secunderabad - 500100

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INTRODUCTION:

Doravirine, chemically it is 3-chloro-5-({1-[(4-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridin-3-yl}oxy)benzonitrile.

The chemical formula is $C_{17}H_{11}ClF_3N_5O_3$. Doravirine has been used in trials studying the treatment of HIV-1, HIV-1 Infection, Renal Impairment, and Human Immunodeficiency Virus (HIV) Infection.

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. Literature surveys reveal few methods for its determination [1-5]. The simple, accurate, precise and validated method for determination of Doravirine was developed by RP-HPLC method. The present study was validated following the ICH guidelines.

MATERIALS AND METHODS:

Materials

Doravirine was kindly supplied from Open Market. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet (PIFELTRO)contains100mg of Doravirine.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loops. The output signals were monitored and integrated using Empower 2 software.

METHODS:

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (0.01N KH_2PO_4 and acetonitrile (50:50 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Agilent C_{18}(250 x 4.6mm x 5 µ) was used for determination. The flow rate was 1.0 ml/minand the column was operated at ambient temperature (~30ºC). The volume of sampleinjected was 10 µL.Prior to injection of the solutions, column was equilibrated for atleast 30min with mobile phaseflowing through the system. The UV detector was set at wavelength of 210nm.

Diluent: Buffer and Acetonitrile (50:50) v/v

Standard Preparation

Stock solution of Doravirine was prepared by dissolving 100 mg in 100 ml volumetric flask add few ml of diluent. Sonicate it for 30min and make up with diluent. Transfer 1ml from the above solution into 10ml volumetric flask to get concentration of 100 µg/ml.

Sample Preparation

About
20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 100mgwas transferred to a 100 ml volumetric flask, dissolved in diluents. Transfer 1ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 100 µg/ml.

METHOD VALIDATION

The developed method was validated as per ICH guidelines [6-8] for its specificity, precision, linearity, accuracy, robustness, limit of detection and limit of quantification by using the following procedures.

System suitability

System suitability and chromatographic parameters were validated such as asymmetry factor,tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Doravirine at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug. The response was found to be linear in the range 25-150 µg/ml for Doravirine.

Accuracy

Accuracy was performed in triplicate for various concentrations of Doravirine equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated.

Precision

A) System Precision

Six standard solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

B) Method Precision
Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

C) Intermediate Precision (Day to Day variability)
Intra day
Two sample solutions have analysed in the same day as per test method conducted the study. For '0' hourand '24' hour, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

Inter day
Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

Limit of detection and Limit of Quantification
LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. LOD and LOQ were found to be 0.20 µg/ml and 0.61µg/ml respectively.

Robustness
Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Doravirine was noted. The factors selected were flow rate and variation in the mobile phase composition.

Assay
The assay & % purity was performed by taking brand PIFELTRO with label claim 100mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form.

Degradation studies
The standard solution injected in the chromatographic system and the standard chromatogram was compared with different stress degradation conditions chromatogram.

RESULTS AND DISCUSSION:

A reverse-phase column procedure was proposed as a suitable method for the determination of Doravirine dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 50:50v/v was used as mobile phase, which showed good resolution of Doravirinepeak. The wavelength of detection selected was 210nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Doravirine was about 2.453minute and none of the impurities were interfering in its assay. The chromatogram of the drug is shown in Fig. 1 and calibration curve is shown in Fig. 2 respectively. The observed peak area values for respective concentrations are shown in Table 1.

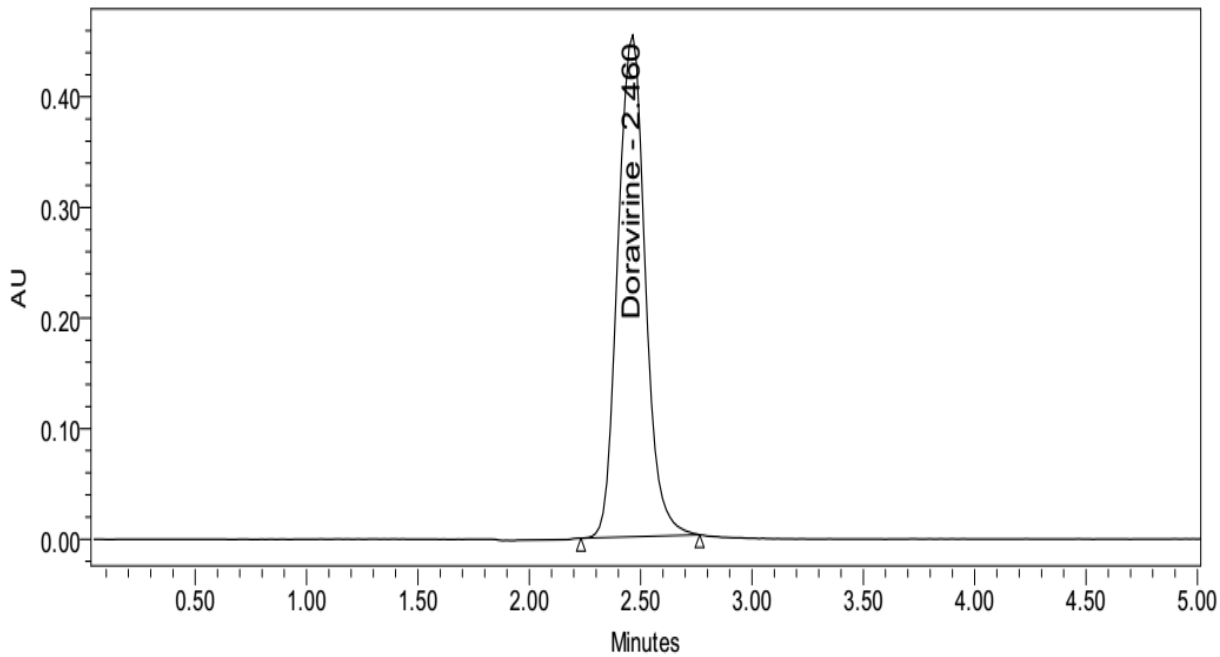

Fig. 1: HPLC Chromatogram of Doravirine

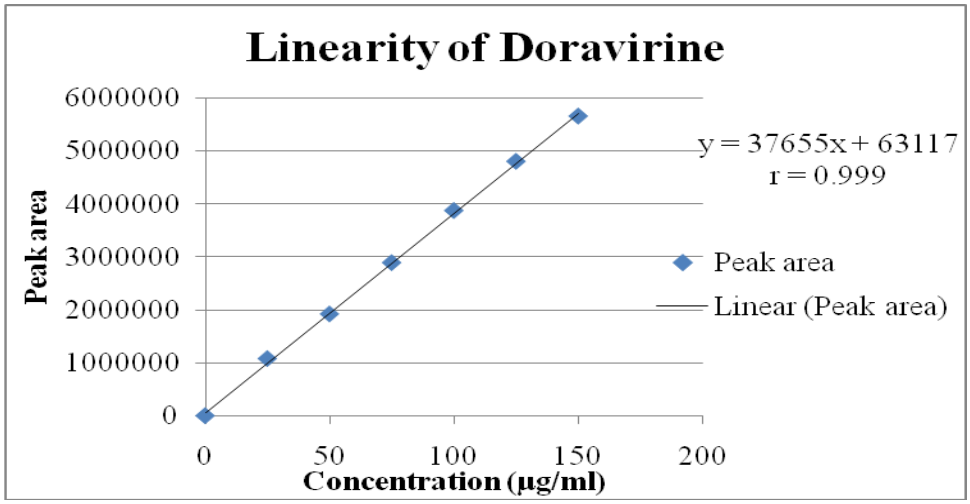

Fig. 2: Calibration curve of Doravirine

Table 1: Calibration curve data of Doravirine

S.No	Concentration (µg/ml)	Injection	Retention time (mins)	Area
1	25	1	2.436	1077583
2	50	1	2.438	1919345
3	75	1	2.434	2888221
4	100	1	2.436	3871778
5	125	1	2.434	4799747
6	150	1	2.437	5653825

The accuracy data, system precision data, method precision data, Intra-day data, and Inter-day data are shown in Table 2, Table 3, table 4, Table 5 and Table 6 respectively. Robustness data relating to change in flow rate and robustness data relating to change in mobile phase composition are shown in Table 7 and Table 8 respectively. Results of analysis of laboratory samples are shown in Table 9. The results for Doravirine of acid degradation studies, base degradation studies, peroxide degradation studies, thermal degradation studies, and UV degradation studies are depicted in Table 10, Table 11, Table 12, Table 13, and Table 14 respectively. Table 15 shows system suitability parameters.

Table 2: Accuracy data

S.No.	Spiked level	Amount Added(µg/ml)	Amount Found(µg/ml)	Average %Recovery*	Std.Dev	%RSD
1(n=3)	50%	25	25.91	99.62	0.05	0.05
2(n=3)	100%	50	50.56	101.12	0.38	0.38
3(n=3)	150%	75	75.89	99.86	0.33	0.33

*n=3 (Average of 3 determinations)

Table 3: System Precision data of 100µg/ml

S.No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	100	1	2.434	3851775
2	100	1	2.456	3870464
3	100	1	2.472	3881727
4	100	1	2.444	3872143
5	100	1	2.498	3862017
6	100	1	2.423	3883187
Mean				3870219
Std.Dev				11932
%RSD				0.31

Table 4: Method Precision data of 100µg/ml

S.No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	100	1	2.428	3874254
2	100	1	2.441	3856875
3	100	1	2.442	3865745
4	100	1	2.447	3875685
5	100	1	2.495	3895745
6	100	1	2.451	3865987
Mean				3872382
Std.Dev				13309
%RSD				0.34

Table 5: Intra-day data relating to change in the day

S.No	Intra-day Precision		
	Peak Area		
	Concentration (µg/ml)	'0'hour	'24' hour
1	100	3874147	3886847
2	100	3898474	3869283
3	100	3869283	3878391
4	100	3898511	3869037
5	100	3875833	3853821
6	100	3871837	3885382
Mean		3881348	3873794
SD		13462	12391
%RSD		0.35	0.32

Table 6: Inter-day data relating to change of day

S.No	Inter-day Precision		
	Peak Area		
	Concentration (µg/ml)	Day – 1	Day – 2
1	100	3864657	3873584
2	100	3872358	3888543
3	100	3859125	3896837
4	100	3883574	3852394
5	100	3856542	3861652
6	100	3887521	3877568
Mean		3870630	3875096
SD		12825	16480
%RSD		0.33	0.43

Table 7: Robustness data relating to change in flow rate (1.0ml/min)

S.No	Flow rate (ml/min)	Average Peak Area*	SD	%RSD
1	0.9ml/min	3872466	5832	0.39
2	1.0ml/min	3862411	4861	0.32
3	1.1ml/min	3881465	3825	0.25

*n=3 (Average of 3 determinations)

Table 8: Robustness data relating to change in mobile phase composition

S.No	Mobile Phase Variation (%)	Average Peak Area*	SD	%RSD
1	M.P-1- (BUFFER: MeOH::51:49)	3881452	4217	0.28
2	M.P-2- (BUFFER: MeOH::50:50)	3872919	3307	0.22
3	M.P-3- (BUFFER: MeOH::49:51)	3850950	3800	0.25

*n=3 (Average of 3 determinations)

Table 9: Results of analysis of laboratory samples (Assay)

Sample	Label	Amount found	% Purity ± RSD*
Brand-1 (PIFELTRO)	100mg	99.98mg	99.98± 0.12

*n=3 (Average of 3 determinations)

Table 10: Results of acid degradation studies of Doravirine

S.No	Doravirine				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	3897636	100	
2	100	24	3890048	92.77	-8

Table 11: Results of base degradation studies of Doravirine

S.No	DORAVIRINE				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	3897636	100	
2	100	24	3806536	93.87	-8

Table 12: Results of peroxide degradation studies of Doravirine

S.No	Doravirine				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	3897636	100	
2	100	24	3821094	94.84	-6

Table 13: Results of thermal degradation studies of Doravirine

S.No	Doravirine				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	3897636	100	
2	100	24	3834604	95.74	-5

Table 14: Results of UV degradation studies of Doravirine

S.No	DORAVIRINE				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	3897636	100	
2	100	24	3876310	98.53	-2

Table 15: System suitability parameters

Validation parameters	Results
Linearity range (µg/ml)	25 – 150
Regression equation	Y = 37655x +63117
Correlation Coefficient(r^2)	0.9998
Accuracy	99.48% to 101.54%
Precision (%RSD)	0.37
Robustness (%RSD)	
Flow rate (0.9ml/min & 1.1ml/min)	NMT 0.39
Mobile phase – Buffer : MeOH(51:49 &49:51)	NMT 0.28
Intermediate Precision (%RSD)	
Intraday – (‘0’hour&‘24’ hour)	NMT 0.51
Interday – (Day 1 & Day 2)	NMT 0.29

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Doravirinein marketed formulation.

CONCLUSION:

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Doravirinein bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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