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ORIGINAL ARTICLE

Development and Validation of UV-Spectrophotometric Method for Estimation of Doravirine

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ABSTRACT
The aim of the study was to establish an easy, accurate and precise UV spectrophotometric technique for quantitative estimation of doravirine in pure and tablet dosage forms. Phosphate buffer (pH 6.8) was used as solvent. Doravirine showed maximum absorbance (λmax) at 230 nm. Linearity was detected in the range of 2.5-15 µg/ml. Linearity equations obtained for doravirine was y = 0.071x + 0.015 with correlation coefficient 0.999. Percentage recovery was found between 98.66% and 99.72%. % RSD for intraday precision and interday precision were detected to be less than two. The LOD and LOQ for doravirine were obtained as 0.28and 0.85µg/ml. The parameters were validated in accordance with the ICH guidelines.
Keywords: Doravirine, UV spectrophotometric method, ICH guidelines, Validation.

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INTRODUCTION

Doravirine($C_{17}H_{11}ClF_3N_5O_3$) is chemically 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. The molecular weight of doravirine is 425.7 g/mol. It occurs as solid in physical state Doravirine is a NNRTI (non-nucleoside reverse transcriptase inhibitor) that is accessible as a single-tablet regimen (STR) with lamivudine and tenofovir disoproxil fumarate (DelstrigoTM) or as PifeltroTM.. Doravirine is approved as preliminary therapy and also for those virologically repressed on a steady antiretroviral (ARV) regimen [1]. It is used to treat HIV-1 infection in grouping with extra antiretroviral therapies (ARTs). It exhibits its antiviral effect through a noncompetitive inhibition of HIV-1 reverse transcriptase. Doravirine has a novel resistance pathway so that it recalls in vitro activity against clinically relevant NNRTI viral mutations K103N, Y181C and G190A. It has a high drug interaction profile contrast with other NNRTIs as it neither induces nor inhibits the cytochrome P450 3A4 (CYP3A4) enzyme[2]. The chemical structure of doravirine was demonstrated in Figure 1. A literature survey on doravirine revealed that UHPLC-MS/MS [3], HPLC–MS/MS [4], HPLC [5,6] methods were reported for the estimation of doravirine. The aim of the present study was to establish an easy, precise and sensitive analytical technique for quantification of doravirine in pure and tablet formulations**.**

MATERIAL AND METHODS

Instruments

The absorbance was measured using a UV-Visible double beam spectrophotometer (UV 1800). The software UV solutions 2.42 was used. For the purpose of measurement, an electronic balance was used. Pipettes and borosilicate glass volumetric flasks were used in the experiment. Microsoft Excel analytical tool 2007 was used to perform all statistical calculations.

Chemicals and reagents

Doravirine sample was attained as gift sample from spectrum pharma research solutions (Hyderabad, India). All the chemicals utilised were of analytical grade.

Preparation of drug stock solution

Doravirine ten mg was precisely measured and transferred to a ten-milliliter volumetric flask. Further, the volume was made up with phosphate buffer (pH 6.8) to attain a drug standard stock solution of 1000µg/ml concentration.

Preparation of working standard solution

1 ml of drug stock solution was transferred to a volumetric flask with a capacity of 10 ml. The volume was then filled with phosphate buffer (pH 6.8) to make a working standard solution with a concentration of 100µg/ml.

Preparation of phosphate buffer (pH 6.8)

In 1000 ml of water, 11.45 grams of potassium dihydrogen phosphate and 28.80 grams of disodium hydrogen phosphate were dissolved.

Preparation of calibration curve

Six volumetric flasks (10 ml) were taken. From drug standard solution 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 ml samples were transferred into the volumetric flasks (10 ml) and diluted with phosphate buffer (pH 6.8) to produce 2.5-15µg/ml. The solutions were scanned from 200-400 nm Uv range using UV-Visible double beam spectrophotometer.

Assay

20 tablets were weighed. In a volumetric flask (10 ml), Powder equivalent to ten mg was transferred into a volumetric flask (10 ml),dissolved in phosphate buffer (pH 6.8). The flask was sonicated for 10 minutes and filtered. Phosphate buffer was used to make up to the number of aliquots of sample solutions in triplicate (pH 6.8). At 230 nm absorbance was measured.

Method validation

Linearity

The ability of a technique to yield test results that are proportional to analyte concentration within a given range is referred as linearity. The working standard solution of doravirine was used to make a series of solutions (2.5-15g/ml) with different concentrations. At 230 nm, the absorbance was measured [7].

Accuracy

The degree to which test results agree with the correct value is referred to as accuracy. By applying an accepted volume of standard stock solution of doravirine to the sample stock solution, accuracy was tested at 50 percent, 100 percent, and 150 percent. The recovery was verified by estimation of drug in triplicate preparations at each specified concentration level and % RSD was calculated[8].

Precision

Precision is characterised as the degree of agreement between individual test results when a technique is subjected to several samplings of a standardised sample. Intraday and interday variance were used to investigate precision. In an intraday study, the concentration of drug replicates was measured three times on the same day. In inter-day study the drug concentrations were analysed on 3 consecutive days which shows the laboratory variation in dissimilar days. Percentage RSD was determined.

LOD and LOQ

The limit of detection refers to the smallest amount of analyte that can be found in a sample. The smallest quantity of analyte in a sample that can be quantitatively estimated with sufficient accuracy and precision is known as the limit of quantification. [9].

LOD and LOQ were determined using the following equation.

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where,

σ = the standard deviation of the response,

S = the slope of the calibration curve.

Robustness

Robustness is an estimate of its capability to remain unchanged by little but intentional changes in parameters of analytical technique and gives a suggestion of its consistency throughout usage. It was performed by changing wavelength (±2 nm) [10].

RESULTS AND DISCUSSION

The established technique was validated according to ICH guidelines. The proposed technique was linear in the series of 2.5- 15µg/ml. Linearity equations obtained for doravirine was found to be y= 0.071x + 0.015with correlation coefficient 0.999. Linearity data was represented in Table 1. Overlain spectra and calibration graph of doravirine were demonstrated in figure 2 and 3. Table 2 shows the results of % recovery for accuracy studies. Mean percentage recovery was found between 98.66-99.72%. The observed data were within the limits indicating good recovery values and accuracy of the developed method. Table 3 and 4 demonstrates % RSD for Precision studies. The RSDs were found to be less than 2% and that indicates that the technique was precise. Robustness data was shown in Table 5.% RSD were found to be 0.13 and 1.23. The data shows that percentage RSD values were well within the limit. The results of all validation parameters were summerised in Table 6.

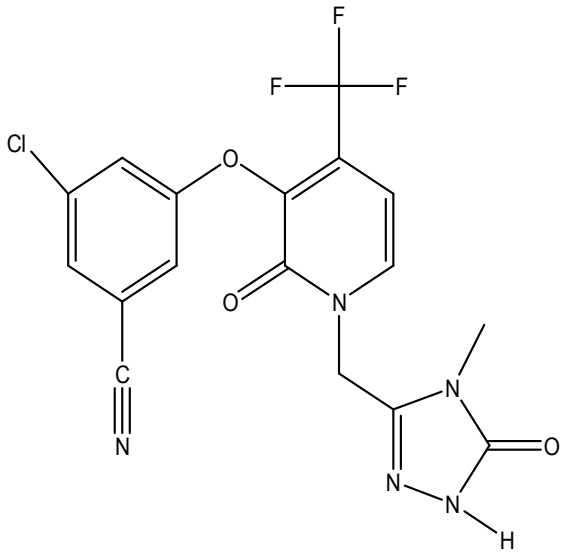

Figure 1: Structure of Doravirine

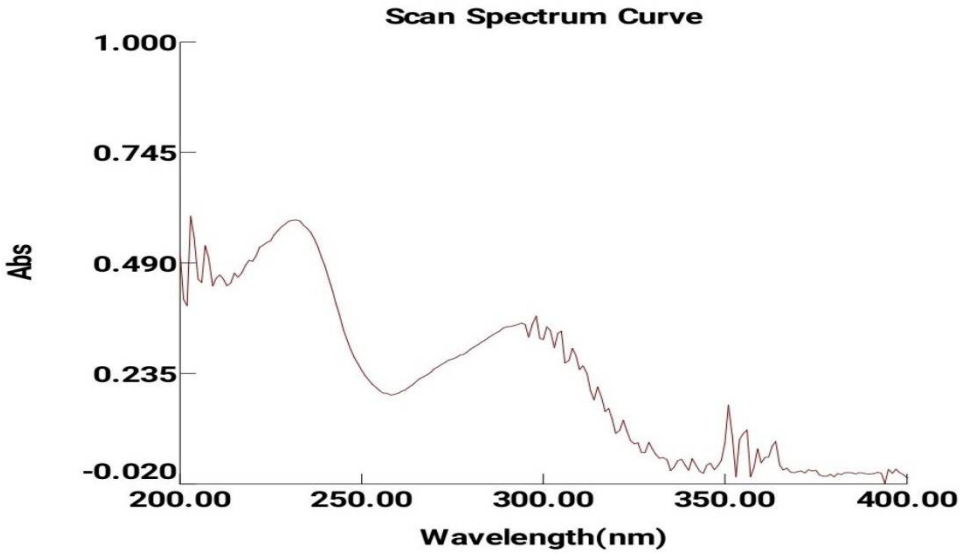

Figure 2: Overlain spectra of Doravirine

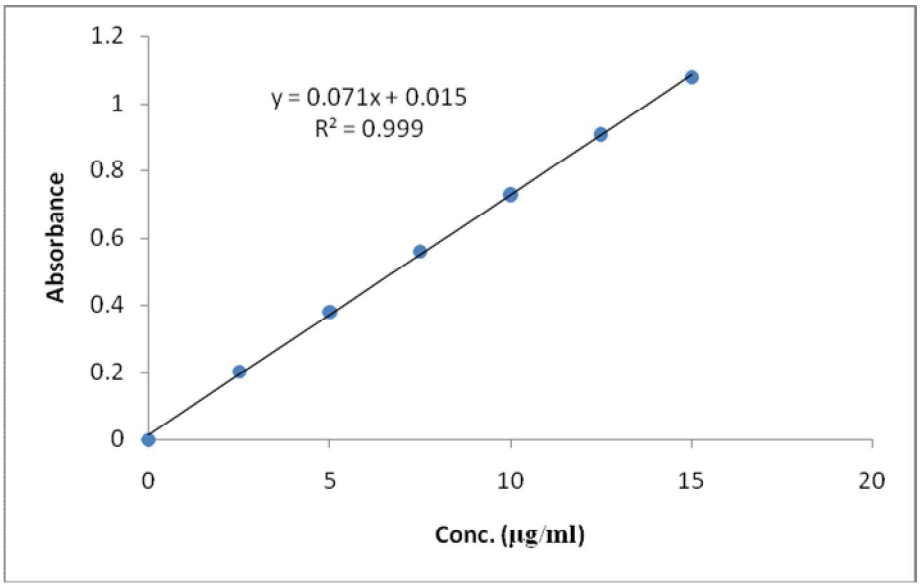

Figure 3: Calibration curve of Doravirine

Table 1: Linearity data of doravirine

Conc.(µg/ml)	Absorbance
2.5	0.200
5.0	0.380
7.5	0.559
10.0	0.727
12.5	0.906
15.0	1.075

Table 2: Accuracy data of doravirine

% of recovery level	Drug in tablet (µg/ml)	Pure drug added (µg/ml)	Drug recovered (µg/ml)	*Recovery (%) ± SD	RSD (%)
50%	10	5	14.99	99.72±0.308	0.309
100%	10	10	19.82	99.09±0.58	0.590
150%	10	15	24.56	98.66±0.38	0.390

*Mean of three determinations at each level

Table 3: Results of Intra-day precision

Concentration (µg/ml)	Concentration found(µg/ml)	*Assay±SD, RSD(%)
5	4.96	99.2±0.27, 0.27
10	9.87	99.12±0.55, 0.56
15	14.84	98.93±0.35, 0.35

*Mean of three determinations at each level

Table 4: Results of inter-day precision

Concentration (µg/ml)	Concentration found (µg/ml)	*Assay±SD, RSD(%)
5	4.91	98.26±0.92, 0.94
10	9.94	99.4±0.75, 0.75
15	14.62	97.79±0.76, 0.77

*Mean of three determinations at each level

Table 5: Robustness data of Doravirine

Wave length (nm)	Concentration (µg/ml)	Absorbance	Wave length (nm)	Concentration (µg/ml)	Absorbance
228	10	0.727	232	10	0.728
	10	0.726		10	0.713
	10	0.728		10	0.729
	Average	0.727		Average	0.723333
	SD (±)	0.001		SD (±)	0.008963
	(%) RSD	0.137552		(%) RSD	1.239109

Table 6: Summary of validation parameters

Parameters	Obtaines values
Maximum absorbance (λ_{max})	230.0
Linearity (µg/ml)	2.5-15
Slope(m)	0.0711x
Intercept(c)	0.0166
Sandells sensitivity (µg/cm^2/0.001)	0.013
Molar absorptivity (L.Mole^{-1}.cm^{-1})	30508.5
LOD (µg/ml)	0.28
LOQ (µg/ml)	0.85
Intra-day precision (%RSD)	0.27-0.56
Inter-day precision (%RSD)	0.75-0.94
Recovery (%)	98.66-99.72
Assay (% w/w)	99.63

CONCLUSION

The method proposed was detected to be easy, specific and precise for the quantification of doravirine in bulk and its tablet dosage forms.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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