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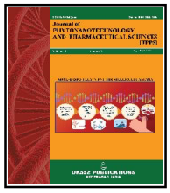

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Creation and validation of a UV spectrophotometric method for the measurement of doravirine in both raw material and pharmaceutical compositions

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Abstract

A straightforward, specific, accurate, precise, and highly sensitive UV spectrophotometric technique was designed and validated for quantifying the drug doravirine in both its pure forms and also in pharmaceutical formulations. The entire method relies on measuring the compound's absorbance at 314 nm using methanol as the solvent. The final linearity of this method spans a concentration range of 1-10 ppm, and it exhibits strong linear correlations with a highest correlation coefficient of 0.999 between absorbance and doravirine concentrations in methanol. Furthermore, the method's LOD and LOQ values were determined. Statistical validation was carried out following the ICH guidelines, which affirmed the precision and accuracy of the method. This validated approach can be efficiently utilized for the regular analysis of doravirine in pharmaceutical dosage forms.

1. Introduction

Doravirine is an antiretroviral drug developed for the treatment of various viral infections like HIV-I infection. Chemically, it is designated as 5-methyl-1-phenylpyridin-2-one, with molecular formula $C_{17}H_{11}ClF_3N_5O_3$ and molecular weight 425.75 g/mol. This substance appears as a white to nearly white powder, exhibiting solubility in organic solvents and limited solubility in water. It possesses a pKa value of 9.6 (Sanchez *et al.*, 2018). Doravirine belongs to the antiretroviral category where it inhibits non-nucleoside reverse transcriptase inhibitor (NNRTI) enzyme which prevents viral transcription and replication (Wilby and Eissa, 2018) (Figure 1).

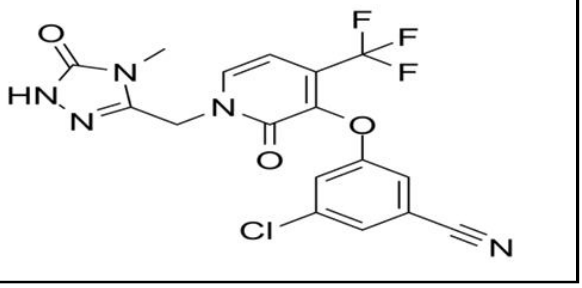

Figure 1: Chemical structure of doravirine.

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As per the literature, it was known that very few analytical methods were identified for doravirine in pharmaceutical formulations such as UV spectroscopic method (Shalin *et al.*, 2011), RP-HPLC methods (Suneetha *et al.*, 2021; Hanuman *et al.*, 2020; Ahamed *et al.*, 2022), UPLC (Tandrima and Shivakumar, 2022) and LC-MS/MS (Ming *et al.*, 2016; Zang and Yang, 2016; Desai *et al.*, 2019). There are some analytical methods that are developed in combination with other drugs (Kokkirala *et al.*, 2021; Godela and Gummadi, 2021; Gollu and Gummadi, 2020). Nonetheless, it became apparent that there was only one UV spectrophotometric method available for assessing doravirine in pharmaceutical formulations. As a result, the current investigation sought to create and validate a UV spectrophotometric approach for quantifying doravirine in both bulk form and pharmaceutical formulations.

2. Material and Methods

2.1 Reagents and chemicals

A gift sample of doravirine working standard was provided by Hetero Labs in Hyderabad. Doravirine tablets (Pifeltro) were acquired from a nearby pharmacy. We sourced all the solvents required for method development from Merck in Mumbai, India. Additionally, all the chemicals employed in the method development were of analytical reagent (AR) graded and were received from Sigma Aldrich in Bengaluru, India.

2.2 Instruments

For the quantification of doravirine in pharmaceutical formulations, a T60V UV-VIS double beam spectrophotometer. The UV Win software was utilized to manage all the instrument parameters. Additionally, other equipment utilized in the study encompassed a digital balance for precise weighing and an ultrasonic bath sonicator.

2.3 Preparing standard solutions and sample solutions

A precisely calibrated 100 mg doravirine reference standard was carefully dissolved in 100 ml of methanol, resulting in a concentration of 1000 µg/ml. Following this, 10 ml of this initial solution underwent dilution with methanol, ultimately reaching a final volume of 100 ml and yielding a concentration of 100 µg/ml. To achieve a concentration of 6 µg/ml, 6 ml of the previously prepared solution was further diluted by adding 10 ml of distilled water.

Twenty Pifeltro tablets were accurately weighed to determine their mean weight. An equivalent of 100 mg of doravirine was then dissolved in 100 ml of methanol solvent. This initial stock solution was subjected to sonication in an ultrasonic bath sonicator for 30 min. Afterward, the solution underwent filtration, and 10 ml of the filtrate was subsequently diluted to a final volume of 100 ml using methanol. Finally, to achieve a concentration of 6 ml, 6 ml of the previously prepared solution was diluted to 10 ml with methanol.

2.4 Method validation (ICH, 2005)

2.4.1 Linearity

The method's linearity was assessed by creating a series of dilutions spanning concentrations from 1 to 10 µg/ml, with subsequent measurement of their respective absorbance values. A concentration-absorbance graph was then generated.

2.4.2 Precision

To ensure originality and avoid plagiarism, you can rephrase the statement as follows: The % relative standard deviation (%RSD) was calculated by conducting precision experiments on the same day (intra-day) and across two consecutive days (inter-day). Six replicates of a 6 µg/ml solution were prepared, and their absorbance values were measured during the designated time intervals.

2.4.3 Accuracy

Method accuracy was assessed by computing % recovery values. Solutions were prepared at three concentration levels (50%, 100%, and 150%) using the standard addition method, and their absorbance was recorded. % recovery was then calculated at each of these three concentration levels.

2.4.4 Specificity

For the determination of the specificity of this method, a blank solution was prepared and observed for any interference of absorbance of solvent with doravirine absorbance.

2.4.5 Detection limit and quantification limit

The determination of the LOD and LOQ involved the utilization of both the standard deviation of the response and also the slope of the regression equation.

3. Results

This investigation sought to create and validate a straightforward and innovative UV spectrophotometric technique for the quantification of doravirine in both bulk form and pharmaceutical formulations.

The UV method was created by performing solubility studies, selection of solvent and selection of detection wavelength by scanning in the range of 200-400 nm as shown in the Figure 2.

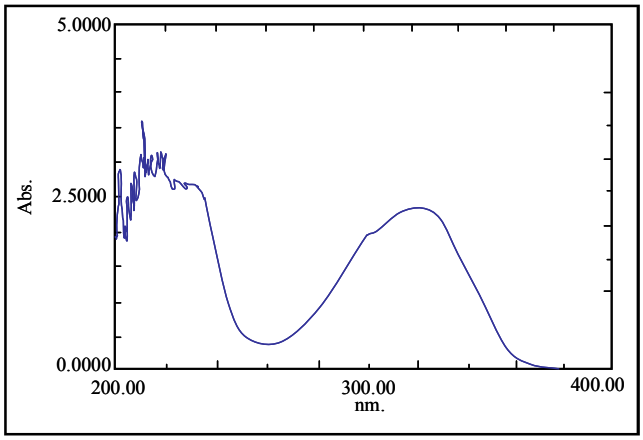

Figure 2: UV spectrum of doravirine.

The optical characteristics of doravirine were presented in Table 1.

Table 1: Optical characteristics

S. No.	Parameter	Result
1	Absorption maximum (nm)	314 nm
2	Linearity range (µg/ml)	1-10 µg/ml
3	Standard regression equation	y=0.0156x+0.0013
4	Slope	0.0156
5	Intercept	0.0013
6	Correlation coefficient (r)	0.9999
7	Molar extinction coefficient ($L.mol^{-1}$ cm^{-1})	7663.5
8	Sandell's sensitivity (µg/cm^2 - 0.001 absorbance units)	0.05
9	Accuracy (% Recovery)	99.80 - 100.62%
10	Precision (Intra-day) % RSD	0.85
	(Inter-day) % RSD	0.77
11	LOD (µg/ml)	0.11
12	LOQ (µg/ml)	0.33
13	Standard error	0.0005

To evaluate the method's linearity, a sequence of dilutions covering the concentration range of 1-10 µg/ml was precisely prepared, and their corresponding absorbance measurements were recorded as shown in Table 2 and plot was presented as Figure 3.

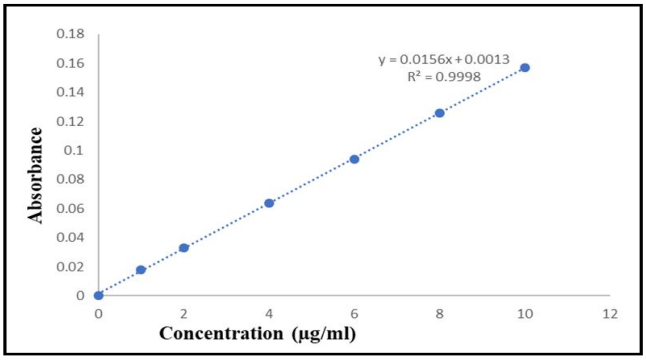

Figure 3: Linearity plot of doravirine.

Table 2: Results of linearity

S.No.	Concentration (µg/ml)	Absorbance
1	1	0.018
2	2	0.033
3	4	0.064
4	6	0.094
5	8	0.126
6	10	0.157
	Regression coefficient (r^2)	0.9998
	Correlation coefficient (r)	0.9999

For the determination of precision, intra-day and inter-day precision studies were performed and presented in Table 3a and 3b.

Table 3a: Intra-day precision results

Sample name	Sample absorbance	% Assay
Sample-1	0.095	98.76
Sample-2	0.096	99.80
Sample-3	0.097	100.84
Sample-4	0.095	98.76
Sample-5	0.095	98.76
Sample-6	0.096	99.80
Average	0.096	99.45
%RSD	0.85	0.85

Table 3b: Inter-day precision results

Sample name	Sample absorbance	% Assay
Sample-1	0.098	99.80
Sample-2	0.097	98.78
Sample-3	0.099	100.82
Sample-4	0.099	100.82
Sample-5	0.098	99.80
Sample-6	0.098	99.80
Average	0.098	99.97
%RSD	0.77	0.77

For the determination of accuracy, % recovery was calculated and presented in Table 4.

Table 4: Accuracy results

Sample No.	Spike level at about (in %)	Amount of doravirine added (mg)	Amount of doravirine found (mg)	% Recovery	Mean % Recovery
1	50	50.00	50.43	100.85	100.15
2	50	50.00	49.37	98.75	
3	50	50.00	50.43	100.85	
1	100	100.00	100.85	100.85	99.80
2	100	100.00	98.75	98.75	
3	100	100.00	99.80	99.80	
1	150	150.00	151.28	100.85	100.62
2	150	150.00	151.28	100.85	
3	150	150.00	150.23	100.15	

4. Discussion

4.1 Solubility studies

Initially, for the development of this method, the standard drug doravirine was subjected to solubility studies where the drug was made to dissolve in different solvents such as methanol, acetonitrile, water, 0.1N HCl and 0.1N NaOH.

4.2 Selection of solvent

Based on the solubility investigations conducted earlier, it was determined that the drug exhibited excellent solubility in methanol. Consequently, methanol was selected as the diluent for the subsequent solution preparations.

4.3 Selection of detection wavelength

To identify the measurement wavelength, a standard solution with a concentration of 10 µg/ml was prepared and subjected to scanning within the UV range of 200-400 nm using a UV spectrophotometer, as illustrated in Figure 2. The maximum absorbance was detected at 314 nm and was subsequently employed for further analysis.

The prepared standard solutions and sample solutions were placed in UV spectrophotometer and their respective absorbance were measured and noted as presented in Table 1.

To evaluate the method's linearity, a sequence of dilutions covering the concentration range of 1-10 µg/ml was precisely prepared, and their corresponding absorbance measurements were recorded. Following this, a graphical representation was constructed by plotting concentration on the x-axis against absorbance values on the y-axis, as depicted in Figure 3, and a summary of the results is presented in Table 2. The correlation coefficient value, obtained from the graph, was calculated to be 0.9999.

The %RSD (relative standard deviation) for intra-day precision experiments yielded a value of 0.85, while for inter-day precision experiments, it was determined to be 0.77. These findings affirm the precision of the developed method. Detailed precision results are available in Tables 3a and 3b.

Method accuracy was assessed through the computation of % recovery. The % recovery ranged from 99.80% to 100.62%, affirming the method's accuracy. A comprehensive summary of the accuracy results can be found in Table 4.

Upon comparing the UV spectrum of the standard solution with the blank solution, no interference was detected in the blank spectrum, confirming the method's specificity.

Furthermore, the method exhibited sensitivity, with a LOD of 0.11 µg/ml and a LOQ of 0.33 µg/ml.

5. Conclusion

We developed a novel UV spectrophotometric method that offers a straightforward approach for quantifying doravirine in bulk materials and formulations. This method underwent comprehensive validation in accordance with ICH guidelines, establishing its accuracy, precision, linearity, specificity, and sensitivity. It can be readily used for routine analysis of doravirine and quality control evaluations in diverse pharmaceutical formulations.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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