RESEARCH ARTICLE

Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-retroviral Drug using UV-spectroscopy

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ABSTRACT

Pharmaceutical analysis plays a vital role in the quality assurance and quality control of bulk drugs. Methods for analyzing drugs in single or multi-component dosage forms can be developed, provided one has knowledge about the nature of the sample, namely, its molecular weight, polarity, ionic character, and the solubility parameter. A simple, rapid, precise, and accurate spectrophotometric method has been developed for quantitative analysis of dolutegravir sodium in tablet formulations. The initial stock solution of dolutegravir sodium was prepared in methanol solvent, and subsequent dilution was done in water. The standard solution of dolutegravir sodium in water showed maximum absorption at wavelength 260 nm. The drug obeyed Beer–Lambert's law in the concentration range of 5–40 µg/mL with the coefficient of correlation (R^2) was 0.9992. The method was validated as per the International Council for Harmonisation (ICH) guidelines. The developed method can be adopted in routine analysis of dolutegravir sodium in bulk or tablet dosage form, and it involves relatively low-cost solvents and no complex extraction techniques. The method can be used to determine the purity of the drug available from various sources.

Keywords: Anti-retroviral drug, Dolutegravir, Linearity, Precision, UV-Spectrophotometry, Validation.

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INTRODUCTION

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative, or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified, as will the procedure for obtaining a representative sample of the material to be analyzed.[1,2]

Dolutegravir is an orally bio-available integrase strand-transfer inhibitor (INSTI). It inhibits human immunodeficiency virus (HIV) integrase by binding to the active site and blocking the strand transfer step of retroviral deoxyribonucleic acid (DNA) integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in viral activity inhibition. If administered orally, it has half-life of approximately 15 hours. The IUPAC name of dolutegravir is (4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a,-hexahydro-2H-pyridol[1',2',4,5] pyrazino [2,1-b][1,3] oxazine-9-carboxamide. Dolutegravir is a white to light yellow powder is slightly soluble in water and is freely soluble in methanol.[3-5]

The present work deals with the development of UV spectrophotometric method and its validation as per International Conference on Harmonisation ICH guidelines. The developed method can be adopted in routine analysis of dolutegravir in

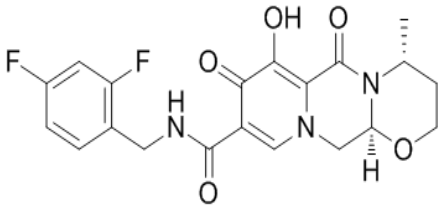

Figure 1: Structure of dolutegravir

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bulk and tablet dosage form, and it involves relatively low-cost solvents and no complex extraction techniques.

MATERIALS AND METHODS

Instrumentation

The analysis was performed on Schimadzu UV-1800, UV/Vis-Spectrophotometer. And other instruments are Schimadzu Digital Electronic Balance-BL 220H, Ultrasonic cleaner, Life Care Equipment Pvt. Ltd.

Chemicals and Reagents

The drug sample of dolutegravir (API) was received as a gift sample from KP labs, Hyderabad, Telangana, India. The formulation was purchased from a local pharmacy, Hyderabad, Telangana. And high-performance liquid chromatography (HPLC) grade methanol, HPLC grade water, and HPLC grade acetonitrile are purchased from Hyderabad.

Selection of Solvent

The solubility of dolutegravir sodium was checked in water, acetonitrile and methanol. It was found to be freely soluble in methanol, slightly soluble in water but insoluble in acetonitrile. Methanol was selected as the solvent for dissolving the drug.[6]

Preparation of Standard Stock Solution

Accurately weighed of dolutegravir working standard equivalent to 10 mg of dolutegravir was transferred into a 100 mL volumetric flask. It was dissolved in 20 mL methanol by sonication for 10 minutes. The final volume was made up to 100 mL with methanol to give the solution containing 100µg/mL of dolutegravir.[7,8]

Selection of Maximum Wvelength (λ_{max})

The standard stock solution was further diluted with water to obtain a concentration level of dolutegravir at 10 µg/mL. The solution was scanned between 200 and 400nm using water as blank.

Preparation of the Calibration Curve

An aliquot of standard stock solution was further diluted with water to get the solutions of concentration within range 5–40 µg/mL. The absorbance was measured at 260 nm against water as blank. All measurements were repeated three times for each concentration.[9]

Assay of Formulation

Twenty tablets were weighed; their average weight was determined and finely powdered. Powder equivalent to 50 mg dolutegravir was accurately weighed and dissolved in a small amount of methanol in 50 mL volumetric flask. Then the volume was adjusted with methanol to obtain the final concentration is 1000 µg/mL. Hence, 10 mL solution was taken and then diluted up to 100 mL with the same solvent in a volumetric flask to obtain the solution of concentration 100 µg/mL. From this solution, an aliquot of 1 mL was diluted to 10 mL using water. The absorbance of sample solution was measured at wavelength 260 nm. This procedure was repeated 6 times.[10,11]

Linearity

Aliquots of standard stock solution were further diluted with water to get the concentration solutions within a range from 5–40 µg/mL. The absorbance was measured at wavelength 260 nm. A linear calibration graph was obtained by plotting the absorbance value versus the concentration of dolutegravir.

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components that may be expected to be present. The specificity of the method for the determination of dolutegravir in tablet dosage form was determined by comparing the spectrum of tablet solution with that of standard solution. The sample spectrum was checked for any interference from the excipients.[12]

Recovery

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80, 100 and 120% level to pre-analyzed samples (10µg/mL) and subsequent solutions were reanalyzed. At each level, three determinations were performed. Accuracy is reported as % recovery, which was calculated from the expression as the equation below.

% Recovery = (Observed value × 100)/True value

Precision

An analytical procedure's precision expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability

The method's repeated repeatability was determined by analyzing six samples of the same concentrations of drug (10µg/mL**)**. Spectra were recorded, and the area of each spectrum was measured.

Intraday and Interday precision (Intermediate Precision)

Intraday precision was determined by analyzing the drugs at three different concentrations (10, 20 and 30 µg/mL**)** and each concentration for three times on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Robustness

The robustness of developed method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and the assay was evaluated. The effect of detection wavelength was studied at ±2 nm.

Solution Stability

The stability of the solution was studied by analyzing the standard solution at 1, 6, and 24 hours intervals.[13,14]

RESULTS AND DISCUSSION

Selection of Maximum Wavelength (λ_{max})

The UV spectrum of dolutegravir has shown maximum absorbance at the wavelength 260 nm. It was selected for the analysis of dolutegravir in bulk and tablet formulation (Figure 2).

Preparation of the Calibration Curve

The calibration curve was constructed by plotting the absorbance against the corresponding concentration. The calibration curve for dolutegravir was represented in Figure 3. The drug was obeyed Beer–Lambert's law in the concentration range of 5–40 µg/mL with a coefficient of correlation (R^2) was 0.9992.

Assay of Dolutegravir Tablets

The amount of dolutegravir present in the formulation was calculated by comparing the absorbance of sample with standard absorbance. Content of dolutegravir in tablet formulation determined by the developed method was in good agreement with the label claim. The results obtained are illustrated in Table 1.

Validation

Dolutegravir showed linear response in the concentration range of 5–40 µg/mL with a correlation coefficient of 0.9992. The spectra obtained from tablet solutions were identical to that obtained from a standard solution containing an equivalent concentration of dolutegravir. This showed that there was no interference from excipients. Therefore, it could be said that the developed method is highly specific.

Table 1: Assay of formulation

Label claim	*Amount found* ± SD (mg)*	*% Label claim*	*% RSD*
50mg	50.18 ± 0.14	100.37	0.28

**Mean of six determinations*

The percentage recovery of standard drug, determined by the developed method at 80, 100 and 120 % of sample concentration, ranged from 98.22 to 99.71%. The values of % recovery and % RSD gave in Table 2 indicate that the method is accurate. The %RSD values for repeatability and intermediate precision were found to be less than 2%. The low % RSD value indicates the precision of the method. The results are summarized in Table 3 and Table 4 respectively.

Assay of dolutegravir for all deliberate changes of conditions was within 98.0–102.0% as reported in Table 5,

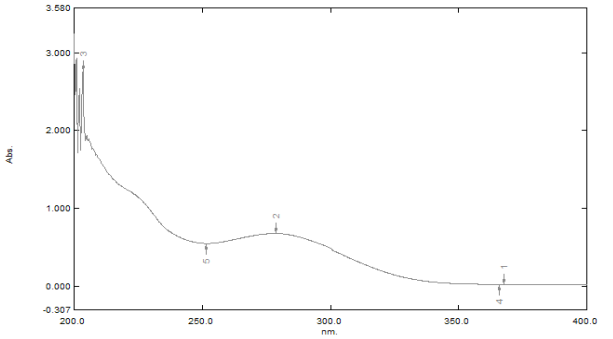

Figure 2: Overlain normal spectra of dolutegravir in methanol

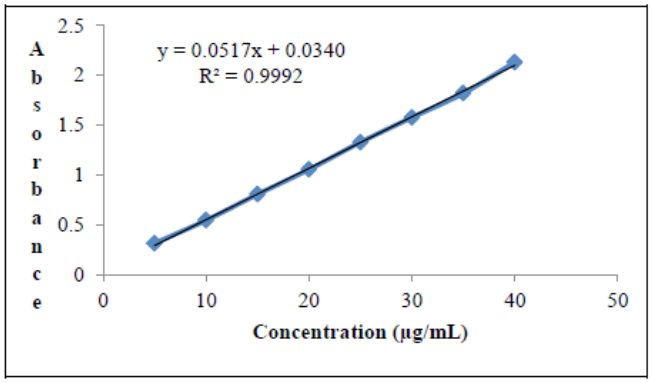

Figure 3: Calibration graph of dolutegravir at 260 nm

Table 2: Results of recovery studies

S. No.	*Level of addition (%)*	*Amount of std drug added (µg/mL)*	*Amount recovered* ± SD (µg/mL)*	*% Recovery*	*% RSD*
1	80	8	7.85 ± 0.13	98.22	1.71
2	100	10	9.97 ± 0.13	99.71	1.29
3	120	12	11.89 ± 0.10	99.09	0.85

**Mean of three determinations, SD- Standard Deviation*

Table 3: Results of repeatability studies

Concentration applied (µg/mL)	*Concentration found* ± SD (µg/mL)*	*% RSD*
10	9.98 ± 0.05	0.54

**Mean of six determinations*

Table 4: Results of intermediate precision studies

	Intra-day precision		*Inter-day precision*	
Concentration (µg/mL)	*Concentration found* ± SD (µg/mL)*	*% RSD*	*Concentration found* ± SD (µg/mL)*	*% RSD*
10	9.98 ± 0.02	0.18	10.05 ± 0.03	0.27
20	19.32 ± 0.15	0.76	19.44 ± 0.16	0.81
30	28.90 ± 0.06	0.20	28.93 ± 0.06	0.19

**Mean of three determinations*

Table 5: Result of robustness studies

S. No.	*Wavelength*	*% Assay* ± SD*	*% RSD*
1	262nm	98.16 ± 0.77	0.77
2	258nm	99.44 ± 0.80	0.80

**Mean of three determinations*

Table 6: Results of solution stability studies

S. No.	*Time (h)*	*% Assay* ± SD*	*% RSD*
1	1	99.02 ± 0.46	0.47
2	6	100.31 ± 0.28	0.28
3	24	99.88 ± 1.33	1.32

**Mean of three determinations*

which indicates the robustness of the method. Meanwhile, results of stability studies indicate that the solution was stable for 24 hours at ambient temperature. The %RSD of assay was 0.65% after 24 hours. The results were represented in Table 6.

CONCLUSION

A reliable and straightforward UV-spectrophotometric method has been developed and successfully validated for the estimation of dolutegravir in bulk and tablet dosage form. The validation test results indicated that the developed method was accurate, precise, robust, and reproducible. This assay system provided an accurate, precise, and sensitive method for dolutegravir quantitation and was successfully applied to the pharmaceutical dosage form. Hence, the developed UV-spectrophotometric method is suitable for routine determination of dolutegravir in the pharmaceutical formulation in quality control laboratories, where economy and time are essential.

The method was validated for parameters like linearity, precision, accuracy, ruggedness, and robustness. The application of this method for the analysis of dolutegravir tablet dosage forms showed that there was no interference of excipients in the determination. The method is advantageous over most of the reported methods in terms of sensitivity, simplicity, cost-effectiveness and experimental conditions. The method does not involve any tedious procedural steps; does not require any extra reagents or longer analysis time, and a very simple instrument are required. The method can be used to determine the purity of the drug available from various sources.

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