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METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF DOLUTEGRAVIR, AN ANTI-RETROVIRAL DRUG USING UV-VISIBLE SPECTROSCOPY

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ABSTRACT

The aim of the present work is to develop sensitive, simple, accurate, precise and costeffective UV-spectrophotometric method for the determination of dolutegravir, an anti-retroviral drug, in bulk and pharmaceutical dosage form; and also to monitor the degradationbehavior of the drug under different stress conditions according to ICH guidelines.The method was developed by checking the solubility of the dolutegravir in various solvents.Dolutegravir is found to be freely soluble in the methanol. The method was developed using methanol and water. The absorbance maxima of dolutegravir using methanol was found to be at 259nm.The method showed high sensitivity of linearity range from 10-60µg/ml with a correlation coefficient (r^2) of 0.9999 .The validation parameters were reported along with Limit of Detection (LOD) and Limit of Quantification (LOQ).The degradation behavior of the drug was studied by subjecting Dolutegravir to an acid and alkalinehydrolysis, oxidative, thermal and UV degradation. This study indicated that Dolutegravir was degraded in different stress conditions

INTRODUCTION

Dolutegravir sodium IUPAC name is sodium (3S,7R)-13-{[(2,4-difluorophenyl) methyl] carbamoyl}-7-methyl- 9,12- dioxo- 4- oxa-1,8 diazatricyclo [8.4.0.0^{3,8}] tetradeca-10,13-dien-11-olate, is a novel integrase strand transfer inhibitor active against Human Immunodeficiency Virus. Dolutegravir (DTG) is active against HIV type 1 (HIV-1) and also has some in vitro activity against HIV type 2 (HIV-2) [1-3]. DTG is a prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV infection in adults and children 12 years of age and older and weighing at least 40 kilograms. DTG is always used in combination with other HIV medicines [4]. It is a discovery of the second-generation integrase stand transfer inhibitor as a result of the collaborative efforts of scientists working for Shionogi (Japan) and GlaxoSmithKline (UK) [5]. Dolutegravir sodium is a white to light yellow powder and is slightly soluble in water. DTG is rapidly absorbed after oral administration. Its low apparent clearance and oral terminal half-life of approximately 13–15 h in healthy volunteers [3] and 11–12 h in HIV-positive adults [6] supports once-daily

dosing without the need for a boosting agent. In total, 34% of the DTG doseis absorbed and excreted with the feces and urine; another 33 –48% is involved in enterohepatic recirculation; and a further portion is secreted in bile [7, 8]. The literature review revealed a liquid chromatography–tandem mass spectrometry method [9] and a sensitive HPLC–MS/MS method [10] for estimation of DTG in human blood plasma has been carried out. There is no UV spectrophotometric method is available for quantitative determination of Dolutegravir in tablet formulation. Further, no official or draft monograph of Dolutegravir sodium was published in any of the pharmacopoeia for compendia applications.

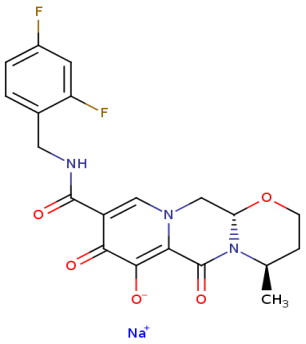

Figure: 1 Chemical Structure of DTG

The present work deals with the development of UV spectrophotometric method and its validation as per International Conference on Harmonization (ICH) guidelines [11-13]. The developed method can be adopted in routine analysis of Dolutegravir sodium in bulk and tablet dosage form and it involves relatively low cost solvents.

MATERIALS AND METHODS

Instrument

Spectrophotometric measurements were carried out using Elico SL 210 UV/Visible spectrophotometer and 1cm matched quartz cells were used for absorbance measurements. Analytical balance is used for weighing the standard and sample.

Materials

Methanol (HPLC grade), Double distilled water was used to prepare solutions wherever required. Hydrogen peroxide (H2O2), hydrochloric acid(HCl) and sodium hydroxide (NaOH) were purchased from Merck (Mumbai, India). Dolutegravir sodium was obtained as gift sample fromMYLAN LABORATORIES (Hyderabad, India). Formulation commercial brand of tablets namely INSTGRA were purchased from local pharmacies and used for analysis.

Reagents

Hydrochloric acid (1M) was prepared by appropriate dilution of concentrated acid with water. A 3% solution of H_2O_2 was prepared by diluting suitable volume of the commercially available reagent to 100 mlwith water in a volumetric flask. Sodium hydroxide solution (1M) was prepared by dissolving required amount of the pellets in water.

Selection of Solvent

The solvent was selected by determining the solubility of Dolutegravir sodium in various solvents namely distilled water, acetonitrile, Methanol. It was found to be freely soluble in methanol, soluble in acetonitrile and slightly soluble in water. Methanol was selected as solvent for dissolving the drug and water is used as diluent for aliquots.

Preparation of Standard Stock Solution

Accurately weighed 10mg of Dolutegravir sodium was transferred into a 10 mL volumetric flask. It was dissolved in 5 mL methanol by sonication for 10 minutes. Final volume was made up to 10 mL with methanol to give the solution containing 1000 µg/mL of DTG.

Preparation of Working Standard Solution

Transfer the 10ml of stock solution to 100 ml volumetric flask and make the volume upto 100 ml by using water as diluent to give the solution containing 100 µg/ml of DTG.

Selection of Maximum Wavelength for Analysis

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

Preparation of calibration curve

Into a series of 10 ml volumetric flasks, aliquots of standard drug solution (0.2–1.8 ml of 100µg/ml) equivalent to 2 - 1.8 µg/ml DTG were accurately transferred and the volume was made up to the mark with water. The absorbance of each solution was then measured at 259 nm against water as blank. Calibration curve was prepared by plotting the absorbance versus concentration of drug. The concentration of the unknown was read from the respective calibration curve.

Assay of Dolutegravir in Tablet

Twenty tablets from commercial brand (INSTGRA) were weighed and crushed into a fine powder. An amount of tablet powder equivalent to 10 mg of DTG was accurately weighed and dissolved in small amount of methanol in 100 ml volumetric flask and then the volume was adjusted with methanol to obtain the final concentration is 100 µg/ml. It was filtered using Whatmann filter paper first 10 ml portion of the filtrate was discarded and a subsequent portion wasused for making aliquots. From this solution, aliquot of 1 mL was diluted to 10 mL using water. The absorbance of sample solution was measured at wavelength 259 nm.

Validation

Method validation was performed in terms of linearity, precision, accuracy, robustness and ruggedness. The method validation was done according to International Conference on Harmonization guidelines for validation of analytical procedures [7-9].

Linearity

Calibration standards of dolutegravir, covering the range 2-18 µg/mL were prepared with the suitable dilution made from dolutegravir working standard solution. The calibration curve was obtained by plotting the intensity of absorbance against of concentration of DTG. The slope and intercept of the calibration line were determined.The limit of detection (LOD) and limit of quantitation (LOQ) are also calculated from the slope.

Precision

The precision of an analytical procedure 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. Precision of the method was determined in terms of repeatability and intraday and interdayprecisions.System precision and method precision were also performed using 10µg/ml standard and sample solution (n=6).

Repeatability: Repeatability of the method was determined by analyzing six samples of 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 (5, 10 and 15 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.

Accuracy: The accuracy of the methods was evaluated by standard addition method. A known concentration standard drug solution was spiked to known concentration of sample solution in three levels (50%, 100%, and 150%) and %recovery is calculated.

Ruggedness and robustness: The robustness of developed method is its capacity to remain unaffected by small changes in conditions. The robustness of the methods was evaluated by measuring the absorbance at different wavelengths whereas the method ruggedness was performed by two different analysts.

Stability Studies

- Forced degradation is the process of subjecting drug compounds to extreme chemical and environmental conditions to determine product breakdown levels and to identify potential degradation products.
- Forced degradation studies may help facilitate pharmaceutical development in areas such as formulation development, manufacturing and packaging, in which knowledge of chemicalbehavior can be used to improve a drug product.
- Forced degradation factors necessary include acid and base hydrolysis, thermal degradation, photolysis, and oxidation.

Forced Degradation Studies (Sample preparation): Acid Degradation Studies:

- 1 ml of working standard solution of drug (10µg/ml) was taken in 10 ml volumetric flask to that add 1ml of 1N Hydrochloric acid was added and kept in oven at 60 ºC for 24hrs .The resultant solution was neutralized with 1N NaOH and diluted with methanol. Then absorbance was measured at 0hrs and 24hrs using methanol as blank.

Alkali Degradation Studies:

- 1 ml of working standard solution of drug (10µg/ml) was taken in 10 ml volumetric flask to that add 1ml of 1N NaOH was added and kept in oven at 60 ºC for 24hrs .The resultant solution was neutralized with 1N HCl and diluted with methanol. Then absorbance was measured at 0hrs and 24hrs using methanol as blank.

Thermal Degradation Studies:

- The standard drug powder was placed in hot air oven at 60ºc for 24 hrs. From standard drug powder a drug solution (100µg/ml) was prepared, from that a concentration of 10µg/ml was prepared and absorbance was measured at 259nm at an interval of 0hrs and 24hrs using methanol as blank.

Photo Stability studies: The standard drug was taken in a petri plate and placed in UV chamber for 24hrs and then prepare 10µg/ml solution from that drug absorbance was checked at 25 using methanol as blank and %degradation was calculated.

Oxidative Degradation Studies: 1ml of working standard solution of standard drug was taken in 10 ml volumetric flask and adds 1ml of 20% v/v H2O2.Solution is kept in hot air oven at 60ºC for 24hrs and then makes up with methanol. Absorbance was measured at an interval of 0 hrs and 24hrs using methanol as blank.

RESULTS AND DISCUSSION

Selection of Wavelength for Analysis

The UV spectrum of DTG has shown maximum absorbance at the wavelength 259nm. It was selected for the analysis of DTG in bulk and tablet formulation (Figure 2).

Fig: 2 Absorbance spectrum of Dolutegravir

Preparation of the Calibration Curve: Calibration standards for ritonavir covering the range of 2-18 µg/mL were prepared and the serial dilutions were made as mentioned in above procedure. The calibration curve was obtained by plotting the absorbance of the dolutegravir versus concentration. The slope and intercept of the calibration was determined. The data are presented in Table 1 and the calibration curve is presented in (Fig 3). Regression analysis of the calibration curve showed a linear relationship between the intensity of absorbance of dolutegravir and theconcentration with correlation. Correlation co-efficient was 0.999 in all the curves assayed in pure form. The values are presented in Table 1.

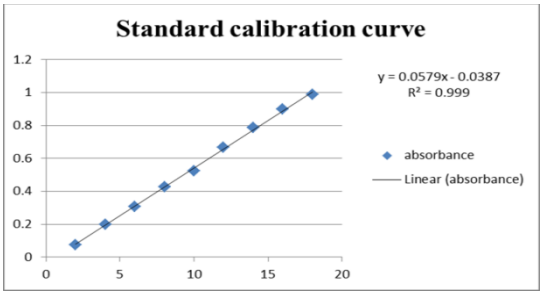

Figure: 3 Calibration curve of Dolutegravir

Table 1: Calibration curve data

Assay of tablets

Concentration(µg/ml)	Absorbance
2	0.075
4	0.199
6	0.305
8	0.425
10	0.525
12	0.665
14	0.781
16	0.898
18	0.989

Commercial TAF tablets were analyzed using developed method and % assay was calculated which was found be to 100.10% using following equation: % assay =AsampleAstdCstdCsample×100 Where, A is absorbance and C is concentration

Parameters	**Dolutegravir**
Measured Wavelength (nm)	259nm
Linearity range	2-18 µg/ml
Slope	0.0579
Intercept	0.0387
Correlation coefficient	0.999
Limit of detection µg/ml	0.217µg/ml
Limit of quantification µg/ml	0.735µg/ml

Table 2: Regression and analytical parameters

Parameter	**%RSD**
PRECISION	
System precision	**0.22%**
Method precision	0.20%
Intra -day (n=6)	0.41% (5µg/ml)
	0.22% (10µg/ml)
	0.36% (15µg/ml)
Inter-day (n=6)	0.56% (5µg/ml)
	0.39% (10µg/ml)
	0.25% (15µg/ml)
Robustness	
At 257nm	0.16%
At 261nm	0.21%
Ruggedness	
Analyst -1	0.22%
Analyst-2	0.26%

Table: 3 Precision, Robustness and Ruggedness

Force degradation study: The stress studies of the drug were carried out by subjecting DTG to acid and alkali hydrolysis, dry heat treatment, UV-degradation and hydrogen peroxide oxidation and later absorption spectra were recorded. The comparison of the absorbance of stressed DTG samples with that of the standard DTG solution showed that DTG has undergone degradation under all stress condition and a summary is given in Table 5.

Table: 4 Accuracy Results

Level	Standard concentration (µg/ml)	Sample concentration (µg/ml)	%Recovery	%RSD
50 %	5	10	97	0.23
	5	10	98.1	
	5	10	96.6	
100 %	10	10	97.7	0.096
	10	10	98	
	10	10	98	
150 %	15	10	103	0.13
	15	10	102	
	15	10	102.6	

Degradation Condition	Time	Absorbance	% Degradation
Acid (1M HCl) at 60˚C	2hrs	0.487	7.11
Base (1M NaOH) at 60˚C	2hrs	0.496	5.44
3% H_2O_2 At 60˚C	2hrs	0.478	8.95
Thermal At 60˚C	2hrs	0.517	1.54
In UV Chamber	2hrs	0.518	1.35

Table: 5 Degradation study results

CONCLUSION

In this study, the degradation behaviour of DTG was studied by subjecting the drug to various stress conditions recommended by ICH. The additional findings in this study show that the drug undergoes an extensive degradation under oxidative (peroxide) condition. The method was validated for parameters like linearity, precision, accuracy, ruggedness and robustness. Application of this method for the analysis of DTG 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; do 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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