METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF DOLUTEGRAVIR IN PURE AND DOSAGE FORMS USING ULTRAVIOLET-VISIBLE SPECTROSCOPY

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ABSTRACT

A colorimetric method for the analysis of dolutegravir in pure form and in tablets has been developed based on the formation of green colour complex. The method is based on the diazotization of carbonyl group and coupling with 3-methyl-2-benzothiazolinone hydrazone reagent in presence of ferric chloride to form green colour complex, by reaction of NH_2 (amine) group present in the 3-methyl-2-benzothiazolinone hydrazone reagent with the carbonyl functional group of dolutegravir by eliminating one water molecule. The complex exhibited absorption maxima at 632 nm obeying Beer's law in range of 10-18 µg mL^{-1}. This method is simple, precise and accurate with recovery of 99.8-100 %. The line equation Y = 0.0082x + 0.0292 with correlation coefficient (r^2) of 0.991 was obtained.

Keywords: Visible Spectroscopy, Dolutegravir, Diazotization, Validation, MBTH

INTRODUCTION[1-7]

Dolutegravir is an HIV- 1 antiviral agent. It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the inhibition of viral activity. Dolutegravir has a mean EC50 value of 0.5 nM (0.21 ng mL^{-1}) to 2.1 nM (0.85 ng mL^{-1}) in peripheral blood mononuclear cells (PBMCS) and MT- cells.

The side effects are headache, nausea, upset stomach, diarrhoea, trouble sleeping, cough, runny nose, skin rashes, unexplained weight loss, persistent muscle aches or weakness, joint pain, numbness or tingling of the hands/feet/arms/legs, severe tiredness, vision changes, abnormal liver function allergic reaction. Chemical structure of dolutegravir is shown in Fig. 1.

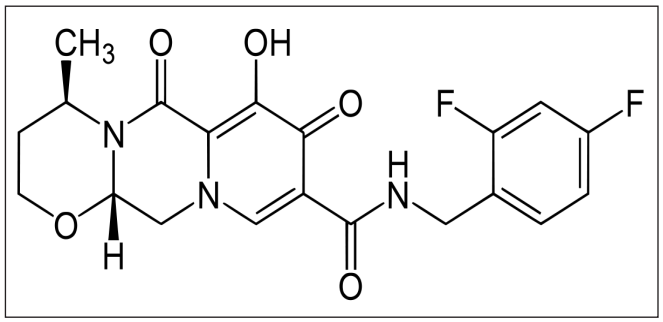

Fig. 1: Chemical structure of Dolutegravir

MATERIALS AND METHODS

Instruments

A SHIMADZU model PHAMASPEC-1800 UV-Visible double beam spectrometer with 1 cm quartz cell was for recording spectra and absorbance measurements.

Materials

Pure drug sample of dolutegravir was kindly supplied as a gift sample by Mylan Laboratories Ltd., Hyderabad, India.

Chemicals and reagents

3-Methyl-2-benzothiazolinone hydrazone (MBTH), distilled water, methanol.

Methods

Preparation of standard stock solution

5 mg of dolutegravir (pure drug) was accurately weighed and transferred to a 50 mL volumetric flask and the drug solubilize using methanol and the volume was made up to 50 mL with distilled water (stock solution). From the above stock solution 10 µg mL^{-1} (1 mL) was pipetted out and transferred to 10 mL volumetric flask. Then, 2 mL MBTH reagent (0.5 %) solution and 2 mL of ferric chloride solution (1 %) were added and the solution

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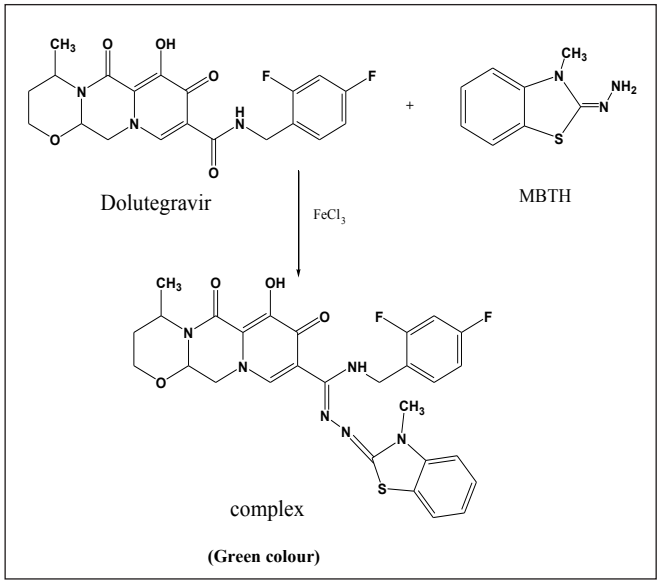

Fig. 2: Reaction between dolutegravir and MBTH reagent

kept for 15 minutes. After that, it was diluted up to the mark with distilled water in 10 mL volumetric flask and green coloured complex was formed. The mechanism of reaction is as shown in Fig. 2. The blank was prepared by adding 2 mL MBTH reagent (0.5 %) solution and 2 mL of ferric chloride solution (1 %) and made up with distilled water.

Assay of marketed formulation

20 Tablets were accurately weighed and finely powdered. An accurately weighed amount of powder equivalent to 0.005 g of dolutegravir was transferred to a 50 mL standard volumetric flask. 10 mL of methanol was added to the flask and mixed thoroughly. The solution was sonicated for 5 min and finally the solution was made up to the mark with distilled water and then filtered through Whatman's filter paper. From the above stock solution, sample solution was pipetted out (16 µg of dolutegravir) was to 10 mL of standard volumetric flask. 2 mL MBTH reagent (0.5 %) solution and 2 mL of ferric chloride solution (1 %) were added to the flask, and the solution kept for 15 minutes and the sample solution were made up to the mark with distilled water. Absorbance was measured using 2 mL MBTH reagent (0.5 %) solution and 2 mL of ferric chloride solution (1 %) and made up to the mark with distilled water as blank. Finally, the concentration of the drugs and percentage purity were calculated[8-10].

Method validation[11-14]

The method was validated using ICH guidelines by determining the following parameters: linearity, accuracy, precision, robustness, ruggedness, precision, detection limit and quantification limit.

Linearity

Five concentrations of the standard dolutegravir (10, 12, 14, 16 and 18 µg mL^{-1}) were prepared and the regression coefficients were determined.

Accuracy

The accuracy of the method was determined by method of standard addition at three percentage levels, namely 50 %, 75 % and 100 %.

Precision

To determine the precision of the proposed method, pure drug solutions (dolutegravir) at a concentration within the working range were prepared and analyzed in three replicates during the same day on three consecutive days.

Robustness

To evaluate the robustness of the methods, the concentration of ferric chloride was changed and the effect of this change on the absorbance of the sample solutions was studied.

Ruggedness

Method ruggedness was evaluated by performing the analysis following the recommended procedure by three different analysts.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ values were calculated to check the sensitivity of the method by using following equations;

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

where σ the standard deviation and S is the slope of the curve.

RESULTS AND DISCUSSION

Selection of wavelength: The detection wavelength was selected by preparing 10 µg mL^{-1} of dolutegravir from the stock solution and scanned in the visible range from 400-800 nm, as shown in Fig. 3.

Assay of marketed formulation: The assay was performed in triplicate and tabulated as shown in Table I.

Table I: Results of marketed formulation by colorimetry

Marketed formulation	Drug	Label claim	Estimated amount (mg)	%purity	% RSD
Instgra	dolutegravir	50mg	50mg	100 %	0.230
			49.8mg	99.6 %	
			50mg	100 %	

Table II: Linearity and range

Sl. No.	Amount taken (µg mL^{-1})	Absorbance (nm)
1	10	0.109
2	12	0.128
3	14	0.147
4	16	0.162
5	18	0.174

relative standard deviation was calculated and are shown in Table III.

Precision: Intra day and inter day precision were determined by evaluating 16 µg mL^{-1} and the percentage RSD was calculated, as shown Table IV.

Robustness: For the proposed method, robustness was carried by modifying the concentration of ferric chloride from 1 % to 0.9 % and 1.1 %, and the results were recorded as shown in Table V.

Ruggedness: To demonstrate ruggedness, three

Table III: Accuracy studies of dolutegravir

Drug	Theoretical % target level	Amount added (µg mL^{-1})	Amount recovered (mg)	% Recovery *mean	% RSD
Dolutegravir	50	8	50	100	0.11
	75	12	50	100	
	100	16	49.9	99.8	

**mean of three readings*

Table IV: Precision studies of dolutegravir

Drug	Amount (µg mL^{-1})	Intra-day		Inter-day	
		% Content	% RSD	% Content	% RSD
Dolutegravir	16	100 99.8 99.8	0.115	100 100 99.6	0.230

Method validation

The overlay spectrum of dolutegravir is given in Fig. 4.

Linearity: The solution obeyed Beer-Lamberts law in the range of 10-18 µg mL^{-1} with regression 0.991, as shown in Table II and Fig. 5.

Accuracy: The recovery studies were carried out three times and the percentage recovery and percentage

different analysts performed the proposed method and percentage RSD was calculated, as shown in Table VI.

Limit of detection (LOD) and Limit of quantification (LOQ): The LOD and LOQ for the method was found to be 1.14 µg mL^{-1} and 3.47 µg mL^{-1}, respectively, indicating the method is suitable for analysing in small quantities, as indicated in Table VII.

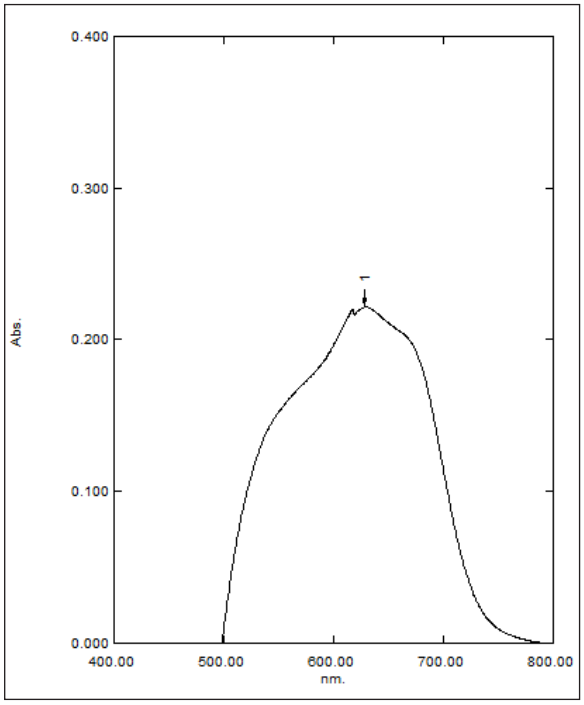

Fig. 3: Visible Spectrum of dolutegravir showing λ_{max} at 632 nm

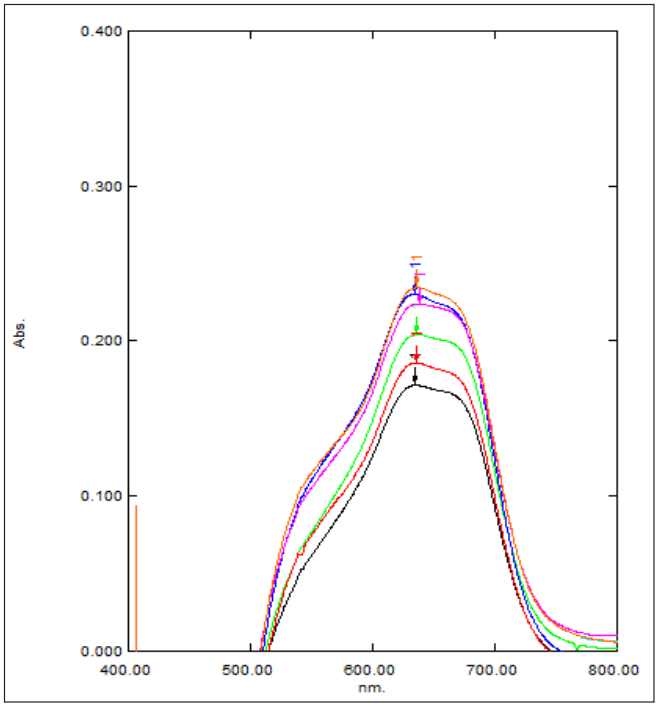

Fig. 4: Overlay spectrum of dolutegravir at 632 nm

Table V: Robustness

Drug	Amount taken (µg mL^{-1})	Parameter altered (concentration of ferric chloride in %)	Amount found (mg)	% Content	% RSD
Dolutegravir	16	0.9 %	50	100	0.230
			49.8	99.6	
			50	100	
		1.1 %	50.1	100.2	0.115
			50	100	
			50.1	100.2	

Table VI: Ruggedness

Drug	Analyst	Amount taken (µg mL^{-1})	Amount found (mg)	% Content	% RSD
Dolutegravir	Analyst I	16	50	100	0.461
	Analyst II		49.6	99.2	
	Analyst III		50	100	

Table VII: LOD and LOQ results

Drug	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Dolutegravir	1.14	3.47

Table VIII: Analytical data

Parameter	Dolutegravir
Detection of wavelength	632 nm
Beer's law limit	10-18 µg mL^{-1}
Regression equation	Y=0.0082x+0.0292
Correlation coefficient	0.991
Slope	0.0082
LOD	1.14 µg mL^{-1}
LOQ	3.47 µg mL^{-1}

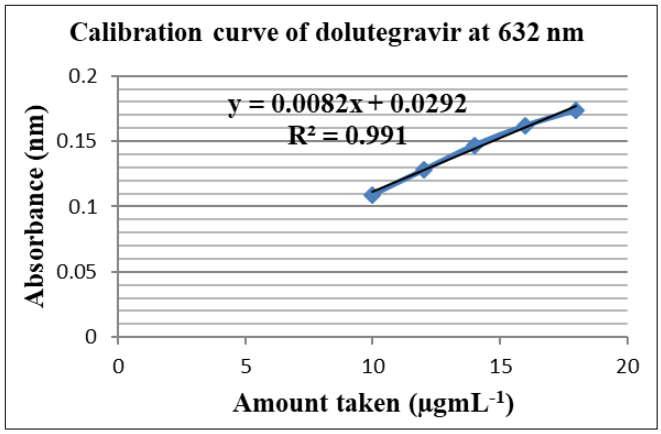

Fig. 5: Calibration curve of dolutegravir at 632 nm

The proposed method for the estimation of dolutegravir in its pure and dosage form can be successfully utilized for routine quality control analysis. Analytical data parameters are shown in Table VIII.

CONCLUSION

A simple, precise, rapid, accurate and reproducible method was developed for estimation of dolutegravir in tablet formulation by colorimetric method. For the determination of dolutegravir by visible spectrophotometric method, 0.5 % MBTH reagent (in 1 % HCl) was used to produce green colored complex. The reaction catalysts used were 1 % ferric chloride solution made up with water. The developed green colored complex showed maximum absorbance at 632 nm.

Linearity was found in the concentration range of 10-18 µg mL^{-1}. The slope, intercept, and correlation coefficient values were found to be 0.0082, 0.0292 and 0.991, respectively. The developed color was stable for about 10 min at room temperature. Low percentage relative standard deviation values show that the developed method is precise, robust and rugged. The recovery studies were carried out at 50, 75 and 100 % levels. The method was successfully used for estimation of dolutegravir in bulk and pharmaceutical formulation.

The calibration curve for the determination of dolutegravir in solid dosage form was found to be precise, selective, rapid, and it can be employed for the routine analysis. It could be precisely quantified and the entire calibration curve shows a linear relationship between the absorbance and concentration. Correlation coefficient was higher than 0.99. The low standard deviation and good percentage recovery indicate the reproducibility and accuracy of the method.

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