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Spectrophotometric quantification of dolutegravir based on redox reaction with Fe^{3+}/1,10-phenanthroline

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Abstract

Background: A simple and sensitive spectrophotometric method was developed for the quantitative measurement of dolutegravir in pure form and pharmaceutical formulation. The present method was based on redox reaction between dolutegravir and ferric chloride, which upon complexation with 1,10-phenanthroline formed an orange-colored complex that showed absorption maximum at 520.0 nm.

Results: The developed method obeyed linearity in the concentration range of 40.00–140.00 μg/mL. The method was also validated as per International Council for Harmonization guidelines and the results were within acceptance values. The validated method was employed for the determination of dolutegravir in pharmaceutical dosage form and the percentage assay value was found to be 102.5, which is in agreement with its label claimed.

Conclusion: The developed redox-based colorimetric method could be used in the routine quality control analysis of dolutegravir present in various pharmaceutical dosage forms.

Keywords: Spectrophotometry, Dolutegravir, Fe^{3+}/1,10-Phenanthroline, Redox reaction

Background

Dolutegravir is chemically 4*R*,(12a*S*)-*N*-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5] pyrazino[2,1-*b*][1,3]oxazine-9-carboxamide and used as second generation HIV-1 integrase strand transfer inhibitor [1, 2]. The structure of dolutegravir was shown in Fig. 1.

Literature review on dolutegravir revealed several analytical methods for its quantification either alone or in combination with other drugs. Ultraviolet-visible spectrophotometric technique for the analysis of dolutegravir sodium in tablet formulation in methanol [3], ultraviolet spectroscopic method using hydrotropic solubilizing agents [4], high performance liquid chromatographic method for its stereoisomers [5, 6], high performance liquid chromatographic and high performance thin-layer chromatographic methods for its salt analysis [7, 8], ultra-performance liquid chromatographic method [9], and bioanalytical methods using high performance liquid chromatography [10] and high performance liquid chromatography-mass spectroscopy [11] were reported in literature. The methods reported for dolutegravir in combination with other antiviral drugs involved reverse phase-high performance liquid chromatographic [12–19], ultra-performance liquid chromatographic [20], and normal phase high performance liquid chromatographic methods using rat plasma [21].

Iron [III] salts play an important role in spectrophotometric quantification of many pharmaceuticals. Ferric form (Fe^{3+}) of iron acts as oxidizing agent and causes oxidation of analyte under study and itself reduces to ferrous (Fe^{2+}) form. The later ions complex with reagent and produces chromophoric complex, which has λ_{max} in visible region. 1,10-Phenanthroline is a heterocyclic

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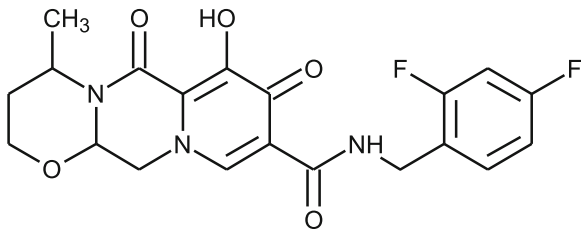

Fig. 1 Structure of dolutegravir

compound and known as a redox indicator, because of its ability to make complexes with various metal ions. Determination of Fe(II) and/or ruthenium (II)-1,10-phenanthroline complexes are well documented in the literature [22–25].

Although many instrumental techniques are available till date, still spectrophotometry plays a significant role in micro/nanogram level analysis of pharmaceuticals. It is simple, low time, and labor-consuming and easy to perform analysis using ultraviolet-visible spectrophotometer. Chromatographic methods, such as high-performance liquid chromatography, high-performance thin-layer chromatography, ultra-performance liquid chromatography, and high-performance liquid chromatography-mass spectroscopy require lavish instrument set-up, skilled operators, expensive solvents, and tedious extraction procedures, unlike colorimetric methods [26, 27]. As to date, no simple colorimetric method was developed for dolutegravir (using Fe^{3+}/1,10-phenanthroline) to the best of our knowledge. In view of the above facts, a simple, sensitive, and extraction-free colorimetric method was attempted for dolutegravir using Fe^{3+}/1,10-phenanthroline as chromogenic reagent. The same with success adopted for the ascertainment of dolutegravir in pharmaceutical formulation.

Methods

Instrument

The method was established by utilizing analytical grade chemicals and reagents. Dolutegravir standard gift sample was provided by Hetero Drugs Pvt. Ltd. and marketed solid dosage form (Tivicay) was procured from local pharmacy. The absorbance of the analytical solutions was determined by using double–beam Shimadzu Ultraviolet–Visible Spectrophotometer 1800. Spectral bandwidth 0.1 nm, wavelength accuracy ± 0.1 nm and a pair of 1 cm path length matched quartz cells were included in it.

Chemicals and reagents

1,10-phenanthroline reagent (0.5% w/v)

The reagent, 1,10-phenanthroline (0.5 g) was accurately weighed and dissolved in sufficient methanol (in a volumetric flask) to produce 100 mL.

Ferric chloride reagent (0.3% w/v)

Ferric chloride (0.3 g) was weighed accurately and dissolved in sufficient distilled water (in a volumetric flask) to produce 100 mL.

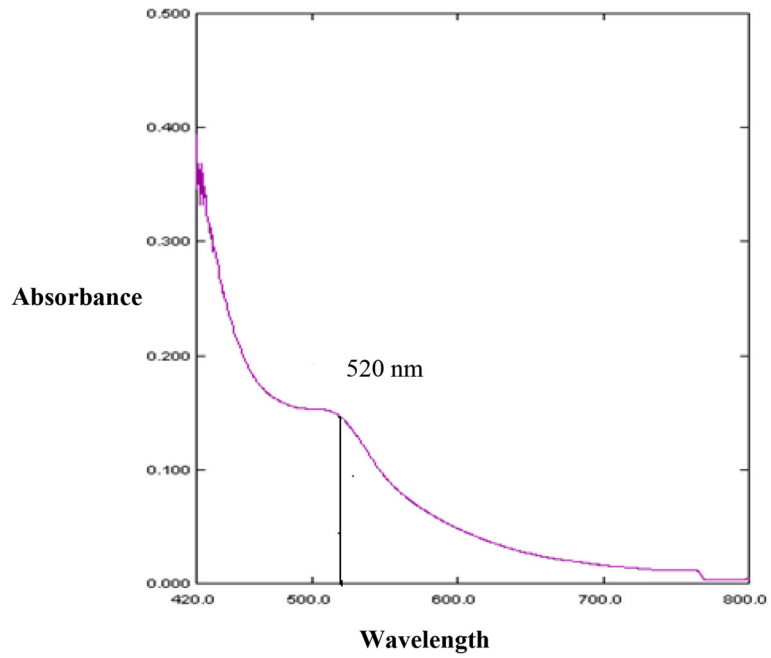

Fig. 2 Absorption spectrum of dolutegravir (30.00 μg/mL) with Fe^{3+}/1,10-phenanthroline

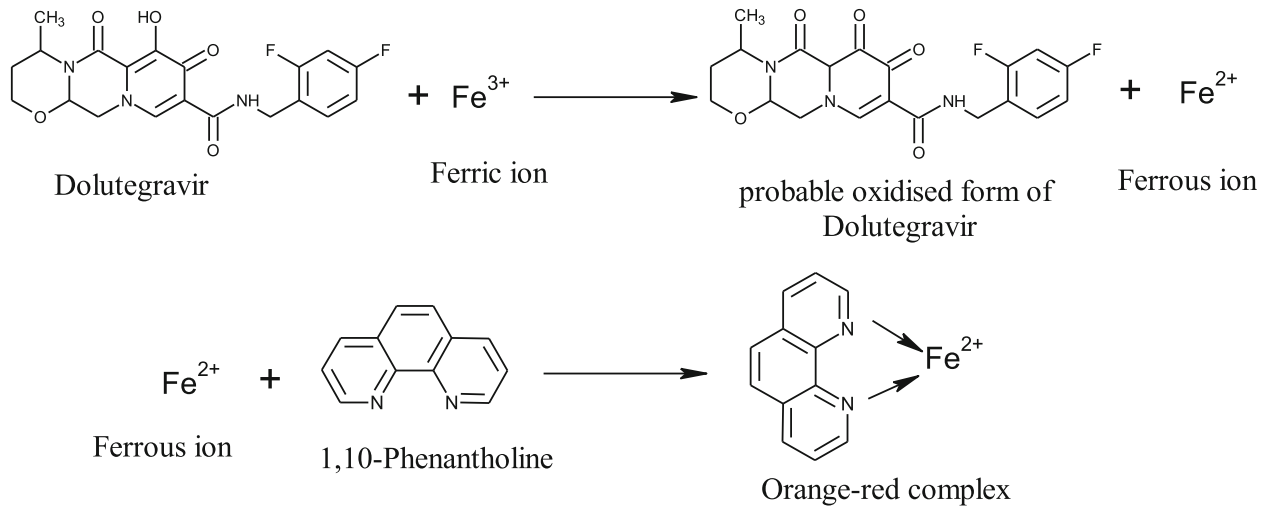

Fig. 3 Probable redox reaction between dolutegravir and Fe^{3+}/1,10-phenanthroline

Dolutegravir standard stock solution

The stock solution of dolutegravir (1000.00 μg/mL) was made by solubilizing 10 mg in 10 mL of acetonitrile and water (1:1). The solution was further diluted with distilled water to get the required concentration of dolutegravir for the λ_{max} determinations and for further analysis.

Analysis of dolutegravir using Fe^{3+}/1,10-phenanthroline

Aliquots of 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL of dolutegravir standard solution (1000.00 μg/mL) were progressively taken in to 10 mL volumetric flasks. To this ferric chloride solution (2 mL, 0.3% w/v), 1,10-phenanthroline solution (1 mL, 0.5% w/v) were added and shaken vigorously and lay aside for 15 min to ensure the color development through redox-coupling reaction. The volume of the volumetric flask was made up to the mark with double distilled water to accord the ultimate concentrations holding 40.00–140.00 μg/mL of dolutegravir.

Blank solutions were made by adopting identical methodology mentioned above, by omitting the corresponding analyte. Then the absorbance of the colored compound was recorded at 520.0 nm against corresponding blank. All measurements were recurrent six-fold for every concentration.

Method optimization

The analytical method was optimized for the reagent concentration (ferric chloride and 1,10-phenanthroline), time for color development and mole ratio of the reaction and the details were provided in next sections.

Method validation

The proof of the method was established based on linearity, accuracy, precision, sensitivity, and robustness according to International Council for Harmonization guidelines [28].

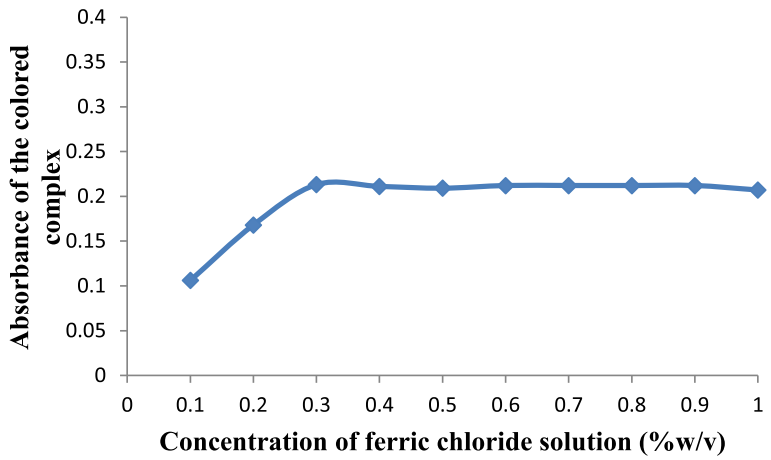

Fig. 4 Effect of concentration of ferric chloride on the absorbance of colored complex at 520.0 nm

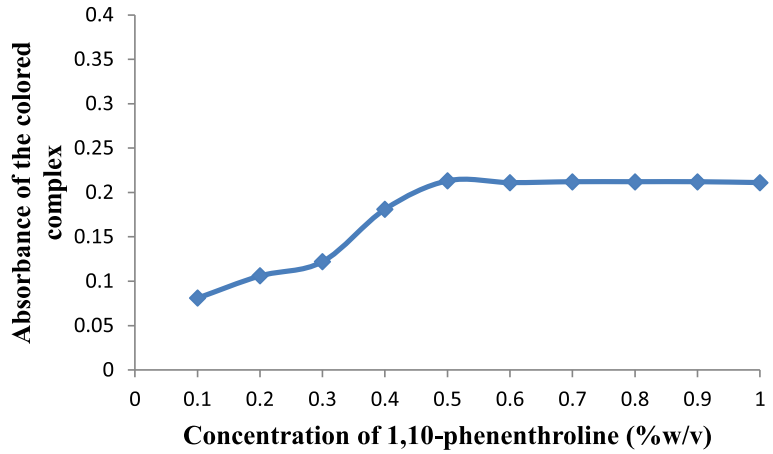

Fig. 5 Effect of concentration of 1,10-phenanthroline on the absorbance of colored complex at 520.0 nm

Linearity

The linearity was examined in pure solutions (n = 6) over the concentration span of 40.00–140.00 μg/mL for dolutegravir. Calibration curve was plotted and from that slope, intercept, and correlation coefficient were computed.

Accuracy

The accuracy of the methodology was decided by recording the recoveries of the analyte using method of standard additions. Distinct levels of standard solutions (80, 100, and 120%) of dolutegravir was spiked to pre-quantified samples and analyzed by proposed method. Each sample was prepared in triplicate at each level. The mean percentage recoveries and percentage relative standard deviation were figured out statistically.

Precision

Precision is the level of repeatability of results as reported between samples analyzed on identical day (intra-day) and samples scampered on three completely distinct days (inter day) in order to examine the intra- and inter-day variant in the method. Solutions accommodating 40.00, 80.00, and 140.00 μg/mL of dolutegravir were subjected to the present spectrophotometric method. The discrepancies in the absorbance of the analyte solutions on intra- and inter-day were deliberately expressed in percentage relative standard deviation.

Sensitivity and robustness

Sensitivity of the method was denoted by limit of detection and limit of quantification values, determined based on standard calibration curve. They were calculated using the formulae 3.3 σ/s and 10 σ/s, respectively, where "σ" is the standard deviation of the y-intercept of the regression equation and "s" is the slope of the calibration curve. Sandell's sensitivity was calculated from the ratio of molecular weight and molar absorptivity of the dolutegravir. Further the

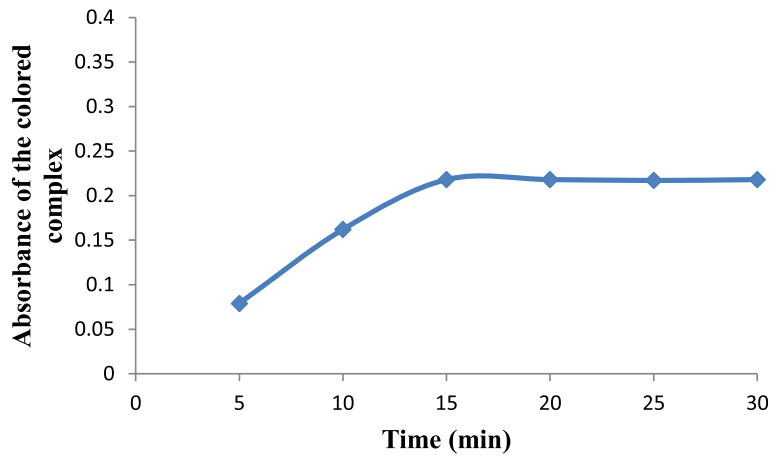

Fig. 6 Effect of time on the stability of colored complex at 520.0 nm

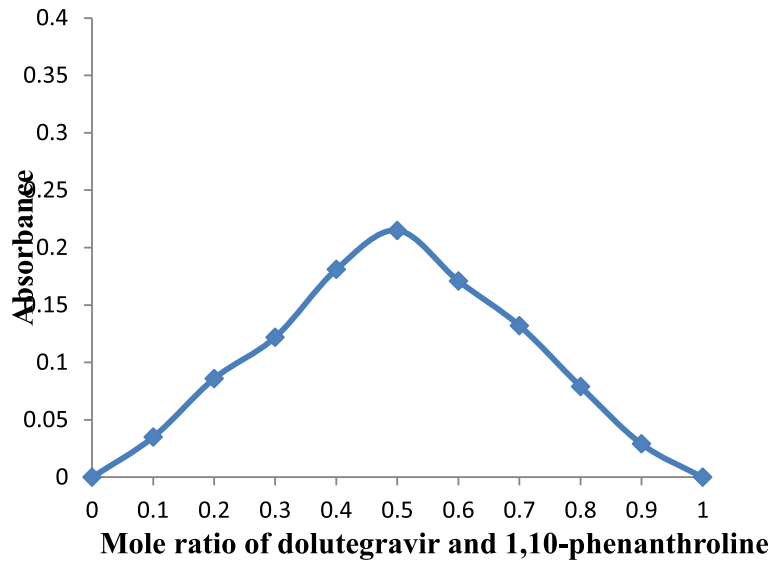

Fig. 7 Job's continuous variation plot for the analytical method

robustness of the analytical method was established by measuring the absorbance of the colored complex by making small changes in the concentration of ferric chloride and 1,10-phenanthroline.

Assay of dolutegravir

Twenty tablets of dolutegravir (Tivicay) were weighed accurately and ground to fine powder. A quantity of powder analogous to 200 mg of dolutegravir was dissolved in acetonitrile and water (1:1), the contents were shaken thoroughly for 5 min. Then, the volume was done up to 10 mL with acetonitrile and water and screened through Whatmann's filter paper (No. 42). To the 1 mL of above filtrate, 2 mL of ferric chloride solution (0.3% w/v), 1 mL of 1,10-phenanthroline reagent (0.5% w/v) were added and shaken vigorously. The emerged solution was diluted up to 10 mL with double distilled water and the colored chromogen was spectrophotometrically measured at 520.0 nm against the corresponding blank.

Results

The reaction of dolutegravir with ferric chloride in the presence of 1,10-phenanthroline resulted in the formation of orange colored product, which showed λ_{max} at 520.0 nm (Fig. 2). The probable reaction mechanism was shown in Fig. 3.

The method was optimized utilizing different concentrations of ferric chloride and 1,10-phenanthroline by varying one factor at a time. The effect of concentration of ferric chloride and 1,10-phenanthroline on the formation of colored complex was studied

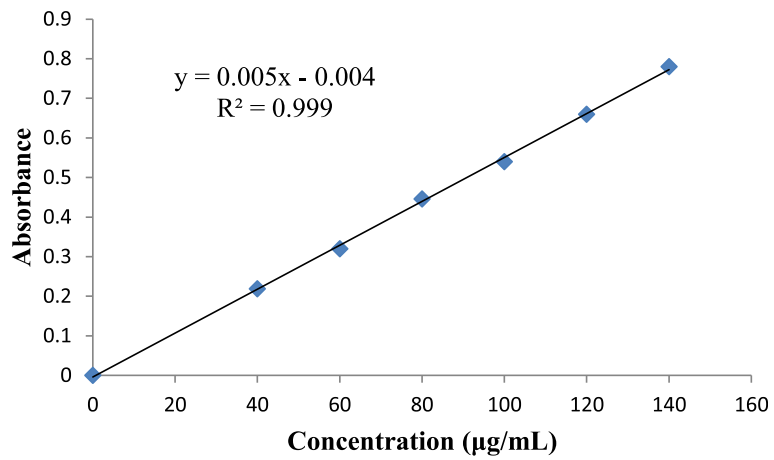

Fig. 8 Calibration plot of dolutegravir

Table 1 Accuracy studies of dolutegravir for developed analytical method

Drug name (brnad name)	Recovery level %	Amount Taken (μg/mL) (A)	Amt of drug spiked (μg/mL) (B)	Total amount of drug (μg/mL) (A + B)	Total amt of drug found[a] (μg/mL)	Amt of drug recovered[b] (μg/mL)	%Amt recovered	%RSD[c]
Dolutegravir (Tivicay)	80	50.00	40.00	90.00	89.73	39.73	99.3	0.69
	100	50.00	50.00	100.00	100.80	50.80	101.6	0.74
	120	50.00	60.00	110.00	112.00	62.00	103.3	0.72

[a]Mean of three determinations
[b]Mean of three determinations calculated with respect to amount of drug spiked (B)
[c]Percentage relative standard deviation

separately by adding 2 mL of different concentrations of ferric chloride and 1 mL of 1,10-phenanthroline by varying one factor at a time. Both were taken at the concentration range of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0% w/v, while the concentration of dolutegravir was fixed at 40 μg/mL. The details were shown in Figs. 4 and 5. Constant and maximum color development was observed with 0.3%w/v of ferric chloride and 0.5%w/v of 1,10-phenanthroline. Hence, the same were considered for the analysis.

The optimum reaction time was determined by monitoring the color development at different time intervals (5, 10, 15, 20, 25, and 30 min). Maximum absorbance values were obtained at 15 min for dolutegravir (Fig. 6). Thereafter, the color developed was stable and the absorbance was constant up to 5 h under optimized conditions.

Stoichiometry of the reaction was studied by continuous variation method. Equimolar solutions of dolutegravir (9.54×10^{-5} M) and 1,10-phenanthroline were prepared by keeping other reaction conditions same as the analytical method discussed earlier. The drug and reagent (1,10-phenanthroline) were mixed in various proportions to produce different mole ratio values (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0). A mole ratio of 0.5 gave the highest absorbance value, which is indicated by the stoichiometric relationship shown in Fig. 7.

The method was further justified as per International Council for Harmonization guidelines to prove its usefulness for quality control analysis of dolutegravir. The developed methodology obeyed Beer's law within the concentration range of 40.00–140.00 μg/mL. Linear regression analysis of the data given the equation $y = 0.0055x - 0.004$ with correlation coefficient 0.999. The relationship between the drug concentration and absorbance is verified from linear regression studies ($r^2 = 0.999$, Fig. 8).

The recovery of the analyte in standard addition method was utilized to establish the accuracy in the method. The percentage recoveries and percentage relative standard deviation were computed and reported (Table 1). The percentage recoveries were varied between 99.7 and 101.8% for dolutegravir and the percentage relative standard deviation values were less than 2.0.

The repeatability and intermediate precision of the method were evaluated using three different levels of dolutegravir (40.00, 80.00, and 140.00 μg/mL). The results were summarized in Table 2 and the percentage relative standard deviation values were found to be satisfactory (< 2.0).

The responsiveness of the methodology was resolute with reference to limit of detection and limit of quantification. The method established 1.52 and 4.60 μg/mL as limit of detection and limit of quantification, respectively and Sandell's sensitivity was found to be 0.182 μg/cm^2 for dolutegravir (Table 3).

The robustness of the proposed method was established by evaluating the influence of the small variations in the concentration of ferric chloride and 1,10-phenanthroine solutions both at 0.3 ± 0.1 and 0.5 ± 0.1% w/v, respectively. The results indicated that these changes did not greatly affect the absorbance of the formed colored complex.

The contemplated method was adopted to estimate the dolutegravir content in marketed formulation (Tivicay). The % assay value for dolutegravir was found to be 102.5

Table 2 Precision studies of dolutegravir for developed analytical method

Conc (μg/mL)	Intra-day		Inter-day	
	Amount found[a] (AM ± SD)	%RSD[b]	Amount found[a](AM ± SD)	%RSD[b]
40.00	39.8 ± 0.0004	0.001	39.6 ± 0.002	0.005
80.00	79.27 ± 0.009	0.011	79.24 ± 0.008	0.01
140.00	140.7 ± 0.10	0.071	140.6 ± 0.12	0.09

[a]Mean of three determinations
[b]Percentage relative standard deviation

Table 3 Optimized conditions in proposed method

Parameters	Dolutegravir
Absorption wavelength (nm)	520.0
Beer's law range (μg/mL)	40.00–140.00
Limit of detection (μg/mL)	1.52
Limit of quantification (μg/mL)	4.60
Correlation coefficient (r^2)	0.999
Slope (m)	0.005
Intercept (c)	− 0.004
Regression equation	$y = 0.005x - 0.004$
Molar Absorptivity (L $mole^{-1}cm^{-1}$)	0.023×10^5
Sandell's sensitivity (μg/cm^2)	0.182

(Table 4). The percentage relative standard deviation value was found to be 0.5 (< 2.0).

Discussion

The present colorimetric technique was rooted on redox reaction between dolutegravir and ferric chloride and further complexation with 1,10-phenanthroline [29]. The method was optimized by considering one factor at a time for the levels of ferric chloride and 1,10-phenanthroline and 0.3% w/v and 0.5% w/v, respectively, were considered as optimum for the analysis. The analytical method was further corroborated for linearity, accuracy, precision, sensitivity, and robustness in line with International Council for Harmonization guidelines. A good linear response between dolutegravir concentration and its absorbance was noticed over a concentration range of 40.00–140.00 μg/mL. The correlation-coefficient value reaching to unity indicated the same. The percentage relative standard deviation values less than 2.0 in recovery and precision studies indicated the accuracy and reproducibility of the method. The developed method was found to be sensitive based on its limit of detection (1.52 μg/mL) and limit of quantification (4.60 μg/mL) values. The validated methodology was employed for the quantification of dolutegravir in marketed formulation. The %assay and percentage relative standard deviation values were within the acceptable limits. Thus, the quantification of dolutegravir in marketed formulation was proved to be fruitful by adopting the proposed analytical method.

Table 4 Data for assay studies of dolutegravir

Drug name	Brand name	Label claim (mg)	Amount found (AM ± SD)[a]	%Assay	%RSD[b]
Dolutegravir	Tivicay	200	205 ± 0.004	102.5	0.50

[a]Mean value of three determinations
[b]Percentage relative standard deviation

Conclusion

The proposed redox-based colorimetric method for the determination of dolutegravir using Fe^{3+}/1,10-phenanthroline as chromogenic reagent was found to be simple, rapid, and does not involve any extraction step. The method was validated for linearity, accuracy, precision, sensitivity, and robustness in line the International Council for Harmonization regulations. The validated method was adopted for the assay of dolutegravir in formulation and results were accorded with the label claim. The results additionally urged that there is no intervention of formulation excipients within the estimation. With these advantages, the proposed methodology can be adopted in routine quality control testing of dolutegravir in its pharmaceutical dosage forms.

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Authors' contributions
AJ had analyzed the samples and completed this work under the supervision of SN and PK. SG helped in experimental work. DAK helped in data analysis and manuscript editing. All authors together contributed for this research work. All authors have read and approved the manuscript.

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Ethics approval and consent to participate
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Consent for publication
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No competing interests to declare.

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