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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DOLUTEGRAVIR BY USING QUALITY BY DESIGN (QbD) APPROACH

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ABSTRACT

Spectrophotometric methods were developed according to Quality by Design (QbD) approach as per ICH Q8(R2) guidelines for estimation of dolutegravir. QbD approach was carried out by varying various parameters, and these variable parameters were designed into Ishikawa diagram. The present work deals with the development of sensitive, simple, accurate, precise and cost-effective UV-spectrophotometric method for the determination of dolutegravir, an anti-retroviral drug, in bulk and pharmaceutical dosage form by UV spectrophotometric method as per International Conference on Harmonisation (ICH) guidelines. The critical parameters were determined by using principal component analysis as well as by observation. Estimated critical parameters in the spectrophotometric method were solvent methanol, wavelength: 260nm, slit width: 0.5, scan speed fast, sampling interval: 0.2nm and proposed method was validated for various parameters like system suitability, linearity, precision, accuracy, detection limits and quantification limits as per the International Conference on Harmonization guidelines ICH Q2(R1). The method's linearity was found to be excellent over the concentration range 5 to 25µg/ml with high correlation coefficient value of 0.999. Limits of detection and quantification were found to be 0.20µg/ml and 0.60µg/ml, respectively. The mean recovery was found to be 100.35% with low percentage relative standard deviation (% RSD) value. The precision study also has shown low % RSD value (<1). No interfering peaks were observed during specificity studies. A simple, rapid, sensitive, accurate, precise and inexpensive spectrophotometric method was developed for estimation of dolutegravirin bulk by using analytical quality by design (AQbD) approach. The same method is also applied for plasma samples study in bioanalytical work.

Keywords: Dolutegravir, Quality by design (QBD), UV-Spectrophotometry, Anti-retroviral drug, Linearity, Validation.

1. 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 [1-3].

Quality by Design approach suggests looking into the quality of analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into final results of analytical process [4]. Concept quality by design (QbD) is used to develop pharmaceutical processes to ensure predefined product quality. QbD concepts are explained in the International Conference on Harmonization (ICH) guidelines Q8 (R1) (Pharmaceutical Development), Q9 (Quality risk management), and Q10 (Pharmaceutical quality system). ICH Q8 (R1) guideline defines QbD as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". QbD is a systematic approach to product development by understanding the effects of various input variables (e.g. process parameters, materials) on the final product (active pharmaceutical ingredient or drug product). Thus, the QbD approach defines appropriate ranges of the input parameters within which the final product's quality is assured. In a similar manner for analytical

methods, the QbD approach involves a full understanding of how the analytical technique attributes and operating conditions affect the analytical performance. Factors to study in analytical quality by design" (AQbD) may include the type of analytical technique chosen, reagents used, and instrumental parameters [5-9].

There are similar advantages of applying QbD principles to analytical methods as to manufacturing processes and product. Several researchers have implemented QbD principles to analytical methods development process. AQbD approach can be used to develop a robust and cost-effective analytical method applicable at any stage of the product's lifecycle. Some regulatory authorities have recently provided the flexibility of changing analytical method without revalidation if the AQbD approach has been implemented during analytical method development. Equivalent to process QbD, the outcome of AQbD is well understood and fit for intended purpose with robustness throughout the lifecycle [10, 11].

The first stage of AQbD approach is to set an analytical target profile (ATP) for the method. ATP defines the goal of the analytical method development process and it is the indicators of method performance. ICH guidelines on validation of analytical procedures, ICH Q2(R1), has given various method performance characteristics for an analytical method. Thus, a QbD based UV spectrometric method can be developed by considering the ICH guidelines Q2 (R1) [12, 13].

The goal of present investigation was to develop a simple, rapid, robust, flexible and economical UV-spectrometric method for the estimation of dolutegravir by using analytical quality by design (AQbD)" approach. For implementing QbD approach to UV-spectro-photometric analytical method, the effect of method input variables on the spectral shape, intensity of absorbance, and absorbance maxima (λ_{max}), were studied and critical parameters were selected for the proposed method. Then proposed method was validated as per the ICH guidelines ICH Q2(R1) [14, 15].

Dolutegravir is an orally bio-available integrase strand-transfer inhibitor (INSTI). 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 HIV replication cycle and results in viral activity inhibition. Dolutegravir is an FDA approved drug for the treatment of HIV infection. If administered orally, it has a half-life of approximately 15 h. The IUPAC name of dolutegravir is (4R,12aS)-N-(2,4-difluorodolutegravirzyl)-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 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. Dolutegravir is always used in combination with other HIV medicines. 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). Dolutegravir is a white to light yellow powder is slightly soluble in water and is freely soluble in methanol. In total, 34% of the dolutegravirdoseis absorbed and excreted with the feces and urine; another 33-48% is involved in enterohepatic recirculation; and a further portion is secreted in bile [16-18]. The structure of dolutegravir is shown in Fig. 1.

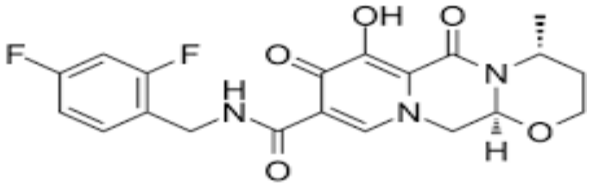

Fig. 1: Structure of dolutegravir

The present work deals with the development of UV-spectrophotometric method for the estimation of dolutegravir and its analytical method validation as per International Conference on Harmonisation (ICH) guidelines. The developed method can be adopted in routine analysis of dolutegravir in bulk and tablet dosage form and it involves relatively low-cost solvents and no complex extraction techniques.

2. MATERIAL AND METHODS

2.1. Instrumentation

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

2.2. 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. All chemicals used were of analytical reagent grade.

2.3. Preparation of stock and working solution

The solubility of dolutegravir in different solvents such as distilled water, 0.01N NaOH, 0.01N HCl, acetonitrile and methanol was determined. The maximum solubility of dolutegravir was found to be in methanol. Hence methanol was used for the preparation of standard stock and working solutions. The stock solution was prepared by dissolving 10 mg of dolutegravir in 20 ml methanol to obtain 100 µg/ml concentration. A standard working solution of 10µg/ml was prepared from stock solution by dilution and used for initial spectral scan in the UV-spectrophotometer.

2.4. Determination of wavelength of maximum absorption

Standard working solution (10µg/ml) of dolutegravir was scanned from 200-400 nm in the UV spectrophotometer to select analytical wavelength. dolutegravir showed maximum absorbance (λ_{max}) at 260 nm (Fig.2). Hence 260 nm was chosen as an analytical wavelength.

2.5. Implementation of AQbD approach in the development of the analytical method

For AQbD approach, Ishikawa diagram was used to study the relationship between variable input parameters and the method performance characteristics of the spectrophotometric analytical methods (Fig.3).

The solvent selection was based on the maximum solubility of dolutegravir. The maximum solubility of dolutegravir was found to be in methanol. Hence methanol was selected as a solvent for a UV-Spectrophotometer analytical method of dolutegravir. For all other variable parameters that are shown in Ishikawa diagram, the absorbance spectrums were recorded by scanning standard working solution (10 µg/ml) in the selected solvent from 200-400 nm in the UV-Spectrophotometer.

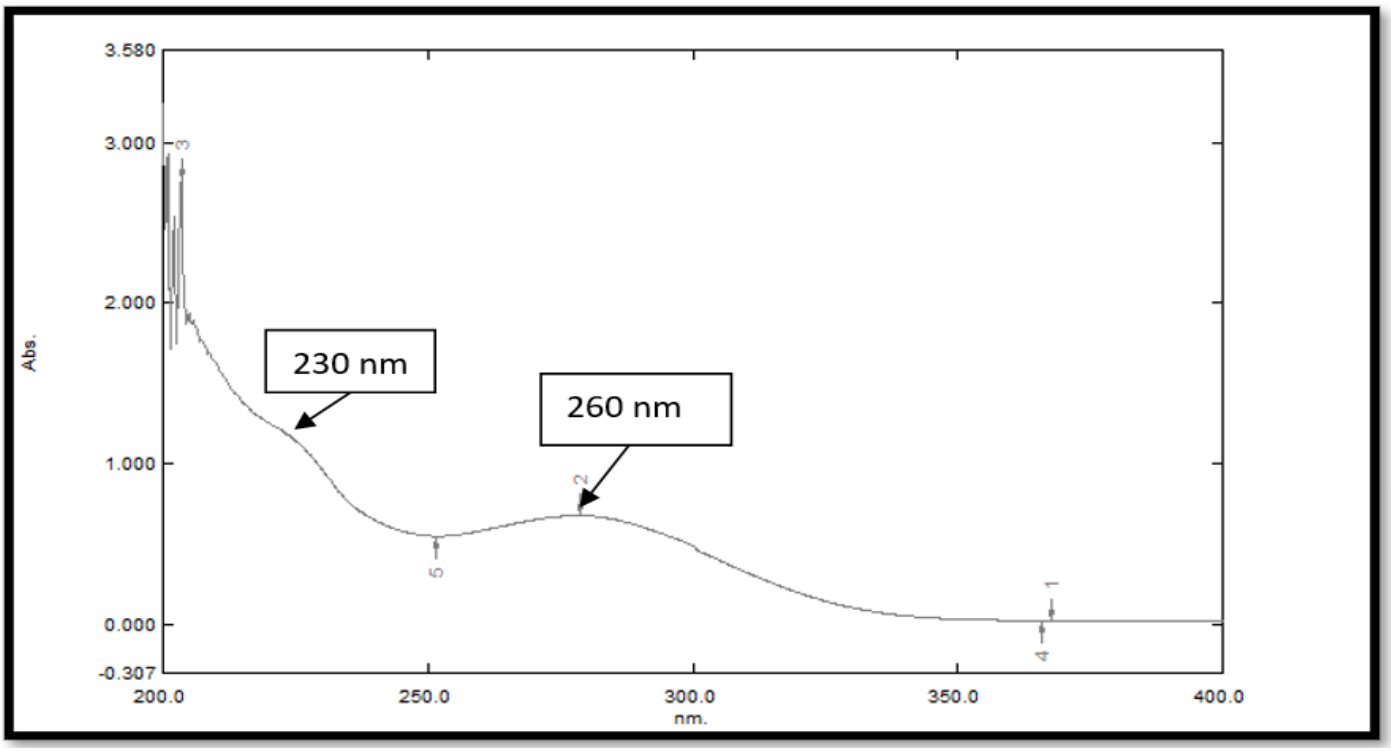

Fig. 2: UV-spectrum of dolutegravir showing maximum absorbance (λ_{max}) at 260 nm

Spectral shape, sharpness, and absorbance intensity of spectrum were recorded and compared at varying scan speeds such as fast, medium, slow, and very slow and varied sampling intervals, such as of 0.1, 0.2, 0.5, 1.0, and 2.0 nm. Thus, indicating no significant changes in spectral shape, sharpness, and absorbance's spectrum due to variation in scan speed and sampling interval.

On the basis of above observation, the critical parameters were selected (Table 1) and by using selected variable parameters, method is further validated as per the ICH guidelines Q2(R1).

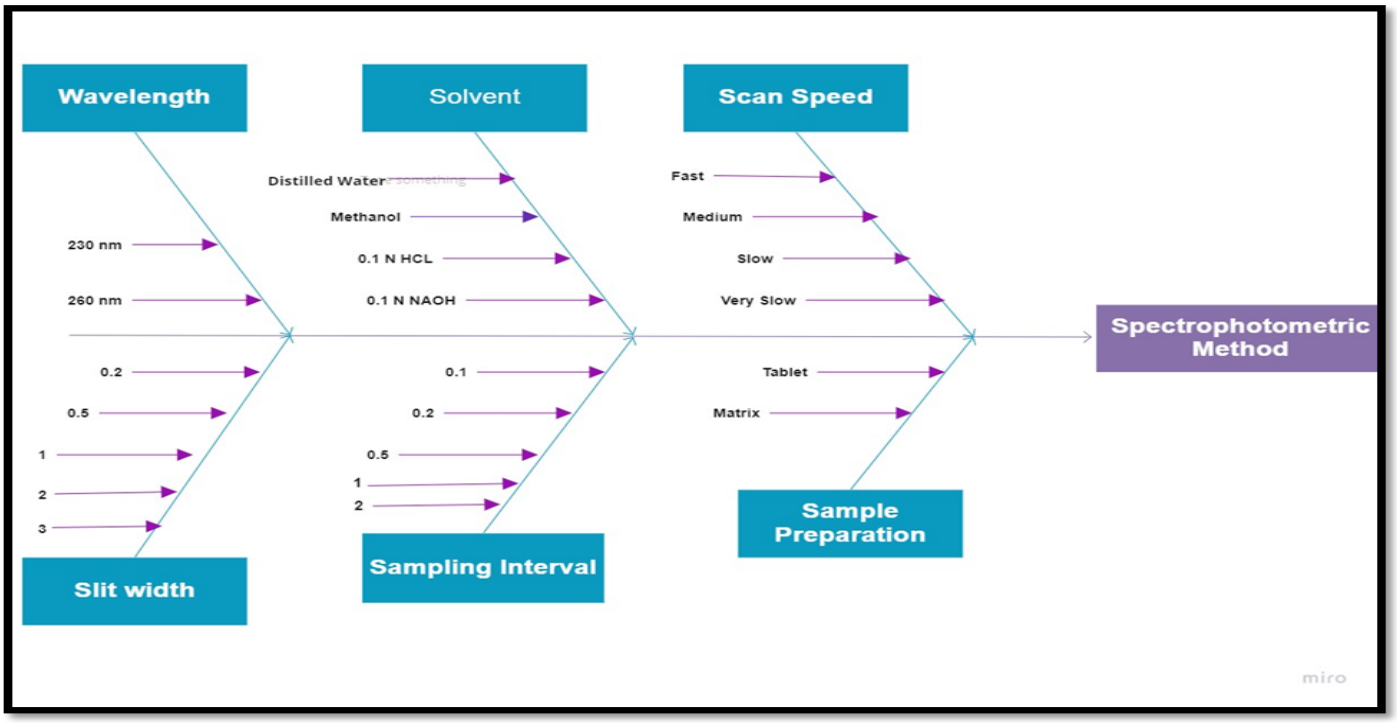

Fig. 3: Ishikawa diagram showing the relationship between variable input parameters and the method performance characteristics of the spectrophotometric analytical methods

Table 1: Critical parameters extractedfor spectrophotometric analytical method of dolutegravir

Parameter	Selected variables
Wavelength	260 nm
Solvent	Methanol
Scan speed	Fast
Sampling interval	0.2 nm
Slit width	0.5

2.6. Validation

The selected critical parameters should comply with the method performance characteristics of an analytical method to achieve the analytical target profile. ICH has laid down various method performance characteristics for an analytical approach in ICH guidelines Q2(R1). Thus, for a spectrophotometric analytical method, it is appropriate to validate the method according to ICH guidelines Q2(R1) on the selected critical parameter to implement the AQbD approach.

Hence by using selected critical parameters, the developed method is further validated as per the ICH guidelines Q2(R1). The characteristics studied were system suitability, linearity, precision, accuracy, specificity, the limit of detection (LOD) and limit of quantification (LOQ).

2.6.1. System suitability

System suitability is done to demonstrate the suitability of the UV-Spectrophotometer system being used for the analysis. Six replicates of standard solution (10µg/ml) of dolutegravir were prepared from a stock solution in the selected solvent (methanol), and absorbance was determined at 260 nm of each replicate using UV-spectrophotometer. Percentage relative standard deviation (% RSD) was calculated for the absorbances.

2.6.2. Linearity

According to the ICH guidelines, an analytical procedure's linearity determines that the test results are directly proportional to the concentration (amount) of analyte in the sample. For linearity study, six solutions of different concentrations (5 to 25µg/ml) were prepared in methanol from a standard stock solution of dolutegravir. Each solution's absorbance was noted at 260 nm in triplicate. The calibration curve was prepared by plotting the absorbance against concentration and % RSD, and the correlation coefficient was calculated by regression analysis.

2.6.3. Precision

According to the ICH guidelines, an analytical procedure's precision determines the closeness of results

obtained by multiple measurements of the same homogeneous sample. Repeatability (intra-day precision) and intermediate precision (inter-day precision) were done to show the precision of the method.

To demonstrate repeatability (intra-day precision) of the test method, six replicates of the 10µg/ml concentration (n=6) were analyzed on the same day. % RSD of assay result of six replicates was calculated. Similarly, for intermediate precision (inter-day precision), six replicates of the 10 µg/ml concentrations were analyzed for assay on three consecutive days and % RSD was calculated.

2.6.4. Accuracy

The accuracy of an analytical procedure shows the closeness of results with the true conventional value. Accuracy was determined by recovery study of dolutegravir. To the known amount of standard solution (10µg/ml), a known amount of standard stock solution was added at a different level, i.e.80%, 100% and 120% to get a final concentration of 15µg/ml, 20µg/ml and 25 µg/ml. Then these solutions were re-analyzed for drug content. Triplicate sets of each level were prepared for the experiment. The recovery of the sample, and % RSD, were calculated.

2.6.5. Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified as an exact value and limit of quantification is the lowest concentration of an analyte in the sample that can be determined with accuracy and precision. Limit of detection and limit of quantification concentrations for dolutegravir were selected based on the residual standard deviation of response and slope method as per ICH guideline. Calibration curve prepared in the linearity study was used for this purpose.

2.6.6. Specificity

According to ICH guideline specificity is the ability to determine the analyte in the presence of possible components, such as impurities, degradants, excipients, etc. To show specificity spectra of standard solution (10µg/ml), diluent and common pharmaceutical excipient (oil and surfactant mixture) were compared.

3. RESULTS AND DISCUSSION

3.1. System suitability

The absorbance of six replicate of standard solution (10µg/ml) of dolutegravir is reported in table 2. For system suitability % RSD of absorbance of replicate solutions should not be more than 2. The results obtained meet the system suitability requirements; this indicates that the system was suitable for the analysis.

3.2. Linearity

The calibration plot of absorbance versus concentration was found to be linear over the concentration range of 5 to 25 µg/ml as shown in table 3 and fig. 5. RSD was found to have a small value of 0.161%, while the correlation coefficient (r^2) has a high value of 0.999 (Table 3). Thus, indicating that test results were directly proportional to the concentration (amount) of analyte in the sample.

3.3. Precision

The reproducibility was determined by repeating the above methods at different time intervals (morning, afternoon, and evening) on the same day (Intraday precision) and three consecutive days (interday precision). The intraday and interday variation for the estimation of dolutegravir was carried out at three different concentration levels of 10, 20 and 30 µg/ml (Table 3).

Table 2: Result of system suitability studies

Replicates of standard solution (10 µg/ml) of dolutegravir	Absorbance at 260 nm
1	0.542
2	0.543
3	0.541
4	0.541
5	0.542
6	0.543
7	0.543
8	0.541
9	0.542
10	0.543
Mean	**0.542**
SD	**0.000876**
% RSD	**0.161**

Table 3: Result of linearity study of dolutegravir

Concentration in µg/ml	Absorbance* mean ± SD (n = 3)
5	0.285
10	0.542
15	0.753
20	0.999
25	1.212

**mean±SD, n=3, SD-standard deviation, RSD-Relative Standard Deviation*

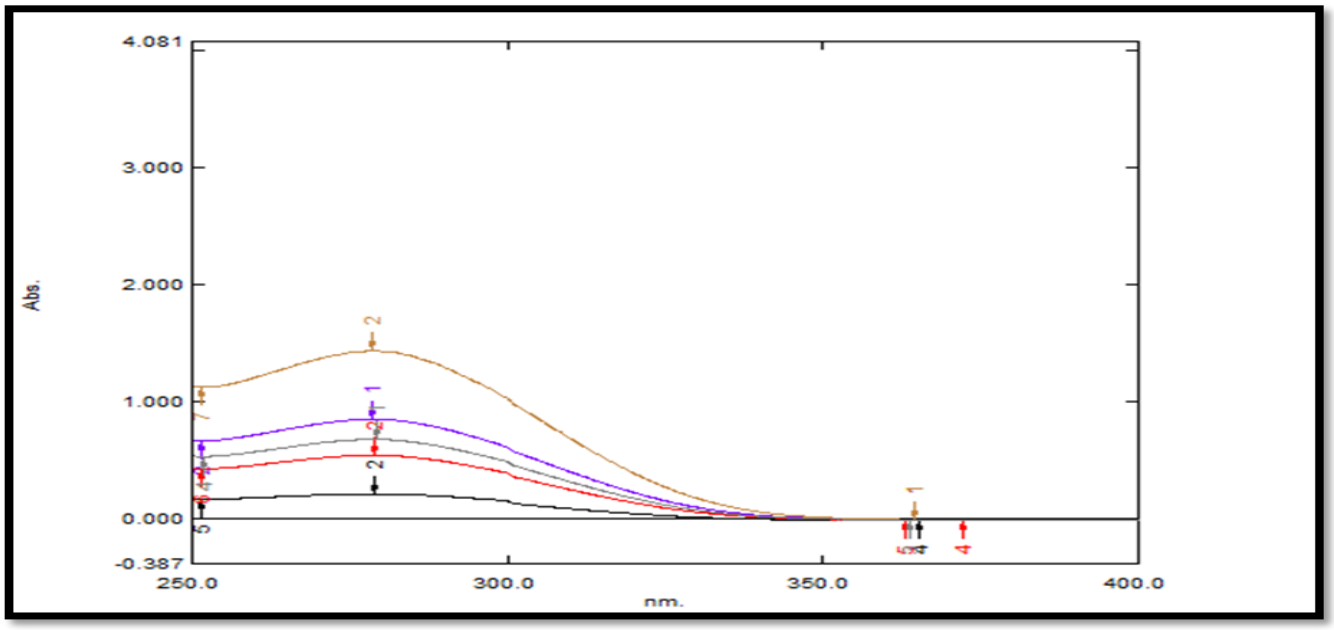

Fig. 4: Overly spectra of dolutegravir

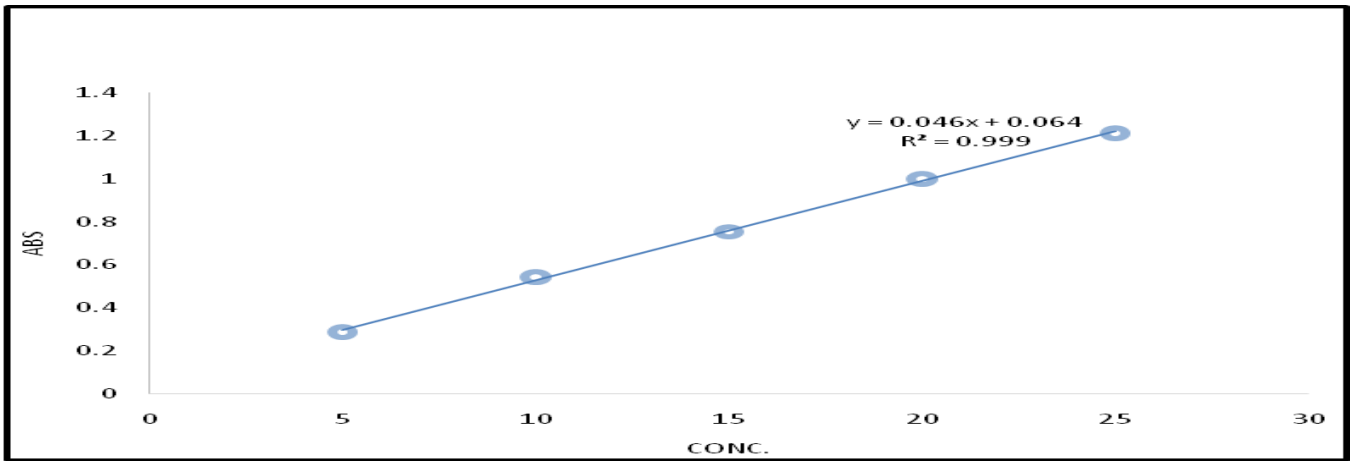

Fig. 5: Linearity spectra of dolutegravir

Table 4: Result of precision studies

Concentration in µg/ml	Intraday (% RSD) (n=3)	Interday (% RSD) (n=3)
10	0.16	0.18
20	0.12	0.15
30	0.14	0.17
Mean	**0.14**	**0.16**

3.4. Accuracy

The purpose of this experiment was to prove the trueness of the assay results obtained by the proposed method. The results of recovery studies are reported in table 5. The mean recovery was found to be 100.35% with low RSD value of 1.28 (<2 %). These results demonstrate the accuracy of the method.

3.5. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was found to be 0.20µg/ml and the LOQ was found to be 0.60 µg/ml. Results are reported in table 6. These results indicated the high sensitivity of the proposed UV method.

Table 5: Result of accuracy studies

Standard solution of known conc. (µg/ml)	Level of standard added (%)	Amount of drug added (µg/ml) (n=3)	Total amount of drug found (µg/ml) * mean±SD (n=3)	Amount recovered (µg/ml)	% Recovery	Mean % recovery of 3 levels (50%, 100%, 150%)	% RSD
10	80	8	17.96	7.96	99.5	99.5	**0.20**
	100	10	19.98	9.98	99.8		
	120	12	21.93	11.93	99.4		

**mean±SD, n=3, SD-standard deviation, RSD-Relative Standard Deviation.*

Table 6: Limit of detection (LOD) and limit of quantification (LOQ) determination

Parameter	Observed value
Slope of regression (S)	0.0462
LOD concentration (µg/ml)	0.1481
LOQ concentration (µg/ml)	0.0488

4. CONCLUSION

A simple, rapid, sensitive, accurate, precise and inexpensive spectrophotometric method was developed for estimation of dolutegravirin bulk by using analytical quality by design (AQbD) approach. On the basis of an investigation of the effect of method input variables on absorbance pattern, the critical parameters have been selected for proposed method and it was further validated as per the ICH guidelines. The developed method does not involve complexity thus has an economic advantage over common chromatographic methods. Therefore, developed spectrophotometric method can be used flexibly and efficiently for the determination of dolutegravir either in bulk or in the dosage formulations. With these advantages, the proposed methodology can be adopted in routine quality control testing of dolutegravir in its pharmaceutical dosage forms. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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Competing interest statement

No competing interests to declare.

6. REFERENCES

1. Rozet E, Ziemons E, Marini RD, Boulanger B, Hubert P. *Anal Chem.*, 2012; **84**:106-112.
2. Akiful HM, Vasudha B, Narender B. *IOSR-Journal of Pharmacy Biological Sciences.*, 2018; **13(2)**:18-22.
3. Priya DS, Sankar DG.*Int. J. Pharm. Sci. Res.,* 2016; **7(7)**:2905-2916.
4. Schweitzer M, Pohl M, Hanna-Brown M. *Pharmaceutical Technology*, 2010; **34(2)**:52-59.
5. Pohl M, Schweitzer M, Hansen Get. *Pharmaceutical Technology Europe.*, 2010; **22(2)**:29-36.
6. Devanna N. *International Journal of Research in Pharmaceutical and Nano Sciences*, 2010;**6(4)**:173-180.
7. Bhusnure OG, Gandge NV, Gholve SB, Giram PS. *Journal of Pharmacy Research*, 2017; **11(5)**:464-471.
8. Garg LK, Reddy VS, Sait SS, Krishnamurthy T, Vali SJ, Reddy AM. *Chromatographia*, 2013; **76(23-24)**: 1697-1706.
9. Bhatt DA, Rane SI. *Int. J. Pharm. Pharm. Sci.,* 2011; **3**:179-187.
10. Bhavyasri K. *Int. J. Pharm. Sci.*& *Scient. Res.*,2019; **5(6)**: 69-74.
11. Reese MJ, Savina PM, Generaux GT, Tracey H, Humphreys JE, Kanaoka E et al. *Drug Metabolism and Disposition*, 2013; **41**:353-361.
12. Shalin K. Parikh, Ankit DP, Dave JB, Patel CN, Sen DJ. *Int J. Drug Dev. &Res.*, 2011; **3(2)**:324-328.
13. Chandrashekar Reddy K, Pavan Kumar KSR, Jagadeesh KV, Srinivas N, Sharma HK, Mukkanti K. *Int J Pharm Pharm Res.*, 2017; **9(2)**:52-63.
14. Shalini SP, Lavakesh Kumar O. *Asian J Pharm Edu Res.,* 2018; **7(4)**:48-56.
15. Mastannama SK, Ananta Sridhar T, Saidulu P. *Int J Pharm Sci Drug Res.,* 2015; **7(4)**:370-375.
16. Dhanwate SS, Pachauri AD, Ghode PD, Khandelwal KR. *J Pharm Sci &Res.*, 2019; **11(8)**:2886-2890.
17. Usha Rani K, Venkateswara Rao P, Srinivasa Rao N. *Int J Res Pharm Chem Anal.*, 2019; **1(2)**:68-74.
18. Castellino S, Moss L, Wagner D. *Antimicrobial Agents and Chemotherapy.,* 2013; **57**: 3536-3546.