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# A Novel UV-Spectrophotometric Method Development and Validation of Dolutegravir In Bulk and Its Laboratory Synthetic Mixture

**SK.Masthanamma[1]*, K.Vasundhara[1], S.Srilakshmi[1], SK.Fathimabi[1]**
*1.Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjunanagar, Guntur–522510, Andhra Pradesh*

## ABSTRACT

Present study describes the spectrophotometric method development and subsequent validation of dolutegravir with greater precision and accuracy. AIDS is the most dreadful disease in the society; this has a very high mortal rate among the countries, as results loosing many beneficial personalities. There are many antiviral and antiretroviral drugs, brought forward by many efficient scientists. A simple, accurate, novel, safe, and precise method could be developed for the estimation of Dolutegravir. Spectrophotometric measurements were carried out using Schimadzu double beam(UV-1800 model) Ultra violet visible spectrophotometer with 10mm matched quartz cells and water as solvent. Linearity for the method was found in the range of 2-14µg/ml ($r^2$=0.997). Tablet formulation was analyzed and % assay for the absorption maxima was found to be 95.6%. Conclusion: The proposed method was validated as per ICH guidelines. Validated studies demonstrated that proposed method is simple, accurate, precise, specific, rapid, reliable, and reproducible.

**Keywords:** Dolutegravir, spectroscopy, dosage form, validation.

*Corresponding Author Email: masthanamma.sk@gmail.com
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# INTRODUCTION

Dolutegravir is a new-generation HIV-1integrase strand transfer inhibition recently approved in EU and japan for the treatment of HIV-1in adolescents and adults in combination with other antiretroviral drugs [1]. Dolutegravir was chemically (RS) (4R,12aS) –N-(2,4-difluorobenzyl)-7-hydroxy-4- methyl-6, dioxo-3,4,6,8,2,12a-hexahydro-2H-pyrido[$1_1$,$2_1$,4,5] pyrazino[ 2,1-b] [1,3] oxazie-9-carboxamide [2].It is slightly soluble in water methanol [1,3]. Dolutegravir is an FDA approved drug for the treatment of HIV infection [4]. On August 13, 2013, dolutegravir was approved by the FDA. On November 4, 2013, dolutegravir was approved by Health Canada [8]. On January 16,2014, Tivicay was approved the European commission for use through the European Union[9]. Dolutegravir is an integrase strand transfer inhibitor (INSTI)[4,5] that does not require ritonavir for cytochrome P450 3AA inhibition, and preferentially blocks the stand transfer step of integration of the viral genome into the host cell´s DNA, which is two –step process mediated by the viral integrase. Like the other approved INSTIs raltegravir(RAL) and elvitegravir (EVG) DTG inhibits the binding of the integrase- viral DNA complex to host cell DNA by chelating Mg+2 ions in the active site. Once integration is blocked, HIV-1 can no longer replicate, and the viral replication cycle is interrupted[5,6]. Dolutegravir has been shown to be more effective than raltegravir, a competitor integrase inhibitor, when treating patients who have already been on ARVs. According to Treatment Action Campaign, raltegravir is currently used in third- line treatment in south Africa, which is used when patients develop resistance to other ARVs. While developed countries will likely use Dolutegravir in first-line. Literature survey revealed that here were no spectrophotometric and chromatographic methods for the estimation of Dolutegravir in bulk and its pharmaceutical formulation. Hence the author made an attempt to develop a simple, economical, enzyme selective, accurate, precise UV spectrophotometric method for the determination of Dolutegravir in bulk and pharmaceutical dosage forms and validated as per ICH guidelines.

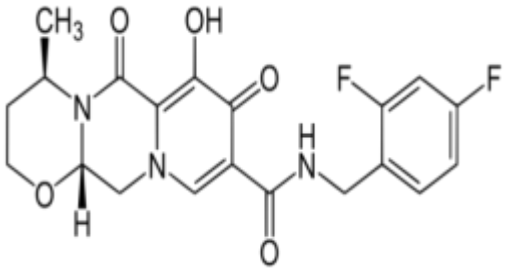


**Figure: 1 Structure of Dolutegravir**

# MATERIALS AND METHOD

**Chemicals and solvents**

Working standard Dolutegravir (99.4%) was obtained as gift sample from Hetero laboratories, Hyderabad, India. Methanol AR grade from Fisheries And double distilled water used for study.

**Instrumentation**

Shimadzu UV-800 double beam spectrophotometer with 1 cm path length supported by Shimadzu UV probe software, version 2.21 was used for spectral measurements with 10mm matched quartz cells. Shimadzu balance (BL -220H) was used for weighing.

**Diluent preparation**

Equal volumes of methanol and double distilled water was mixed and sonicate for 10 min.

**Preparation of standard stock solution**

Working standard Dolutegravir 10 mg was weighed accurately and transferred to a 10 ml volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000µ g/ml. it was further diluted with distilled water to get the concentration of 100 µg/ml. from this solution a series of aliquots were prepared for further method development.

**Method development**:

For the selection of analytical wavelength 10 µg/ml solution of Dolutegravir was prepared by appropriate dilution of standard stock and scanned in the spectrum mode from 200nm to 400nm. From the spectrum $\lambda_{max}$ of Dolutegravir,258nm was selected for the analysis was shown in figure 2 . The calibration curve was prepared in concentration range of 2-14µg/ml a 258nm. The calibration curve for Dolutegravir was plotted in the concentration v/s absorbance was shown in figure no.3 and regression equation was calculated for the determination of amount of Dolutegravir in synthetic mixture.

**Estimation of Dolutegravir in synthetic mixture:**

For estimation of Dolutegravir synthetic mixture was prepared with Dolutegravir API and excipients (Mannitol, Microcrystalline cellulose)with the strength of 50 mg of Dolutegravir in glass motor and pestle, after proper mixing, weigh accurately about a quantity of powder which was equivalent to 10 mg of Dolutegravir was transferred to 10 ml volumetric flask and dissolved in and make up the final volume with distilled water to

obtain a sample stock solution of 1000µg/ml of Dolutegravir [7] .It was filtered with Whatmann filter paper no.41; from this solution required test concentration was prepared by appropriate dilution. The concentration in the test solution was estimated at 258nm. Results of laboratory mixture was shown in Table 1. The assay procedure was repeated 6 times(n=6).

**Method validation:**

The method was validated according to ICH guidelines to study accuracy, linearity, precision, LOD, LOQ.

**Linearity**

In order to find out linearity range of proposed UV spectrophotometric method, studies were carried out by plotting absorbances of analyte against concentrations of the analye. A good linear relationship ($R^2$=0.997) was observed between concentrations of Dolutegravir and the corresponding absorbance. The regression equation of Dolutegravir concentration its absorbance was found to be y= 0.068x+0.003,(where y is the absorbance and x is the concentration of Dolutegravir).The slope, intercept and the correlation coefficient of the drug were shown in Table 2.

**Accuracy**

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out in different recovery levels (50%,100%and150%) by adding placebo to the pre analyzed formulation. The solutions were suitably diluted in the range and then each of the dilution was observed 6 times. The % recovery of drug was found to be 95.6%. The results were shown in the Table.3.

**Precision**

Precision is the level of repeatability of results as reported between samples analyzed on the same day (intra-day) and samples run on 3 different days (inter- day) to check the intra-day and inter-day variation of the method, solution containing 4, 6and 8µg/ml Dolutegravir were subjected to the proposed spectrophotometric method of analysis and the recoveries obtained were noted. The precision of proposed method i.e. the intra and inter-day variations in the absorbance of the drug solution was calculated in terms of %RSD and the results were presented in the Table 4. Statistical revealed that relative standard deviation of drugs at different concentration levels for times was less than 2.0

**LOD**

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte.

The standard deviation and response of the slope

LOD=3.3 standard deviation (σ)/s.

**LOQ**

The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy.

The standard deviation and response of the slope

LOQ=10*standard deviation (σ)/s.

## RESULTS AND DISCUSSION

For quantitative estimation of dolutegravir in bulk and laboratory synthetic mixture a validated methods was proposed ,the absorbance maxima was found to be 258 nm(figure.2) and linearity of drug was shown in figure no.3.The %assay was found to be 95.6%(Table 1). No interference was observed from the pharmaceutical excipients. The % recovery obtained was 95.6%(Table 3) the proposed are very precise, the %RSD is less than 2. Hence the proposed method was validated in terms of linearity, accuracy and precision. The present work provides an accurate and sensitive method for the analysis of Dolutegravir in bulk and its tablet formulation. The UV spectrophotometric method was developed and validated as per ICH guidelines. Hence the developed spectrophotometric method was accurate, precise and can be employed successfully for the estimation of Dolutegravir in bulk and its formulation.

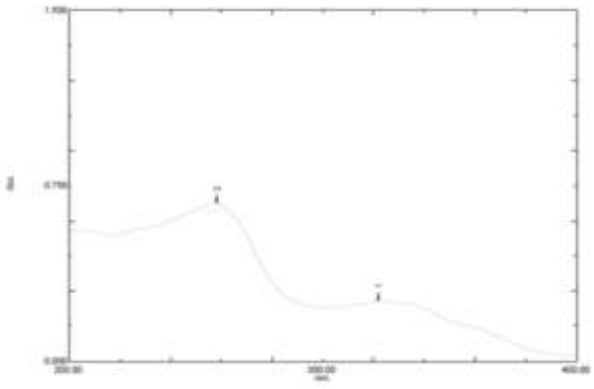

**Figure: 2 Overlay Spectrum of Dolutegravir**

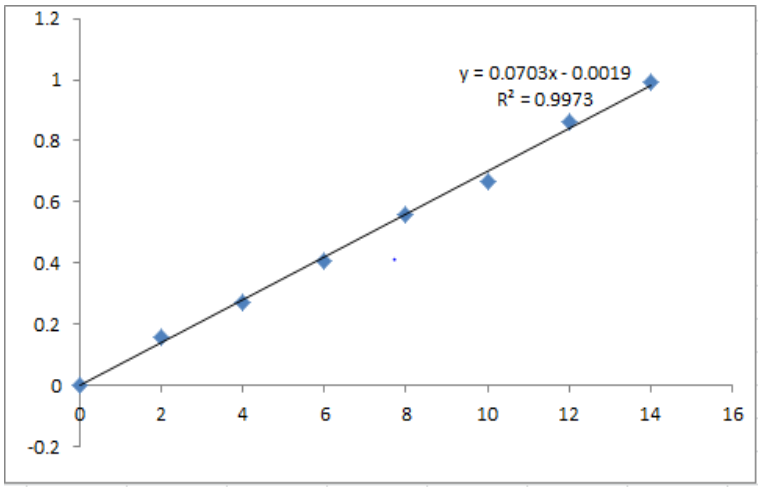


**Figure: 3 Calibration curve for Dolutegravir**

**Table 1: Results of laboratory synthetic mixture**

| S.no | Test concentration(µg/ml) | Amount found (µg/ml) | % Assay |
|---|---|---|---|
| 1. | 4 | 3.89 | 97% |

**Table 2: Optical characteristics of the proposed method**

| S.no | Parameter | Results |
|---|---|---|
| 1. | Linearity | 2-14 µg/ml |
| 2. | Linearity equation | Y= 0.068x+0.003 |
| 3. | Slope | 0.068 |
| 4. | Intercept | 0.003 |
| 5. | Correlation coefficient | 0.997 |
| 6. | LOD(µg/ml) | 0.12 |
| 7. | LOQ(µg/ml) | 0.38 |

**Table 3: Recovery studies of proposed method**

| S.no | Preanalysed concentration (µg/ml) | % Recovery level | Amount added(µg/ml) | Amount found (µg/ml) | % Recovery |
|---|---|---|---|---|---|
| 1. | 4 | 50 | 2 | 6.07 | 101 |
| 2. | 4 | 100 | 4 | 7.51 | 94 |
| 3. | 4 | 150 | 6 | 9.25 | 92 |

**Table 4: Precision studies of the proposed method**

| | INTER DAY | | | INTRA DAY | | |
|---|---|---|---|---|---|---|
| S.no | Concentration (µg/ml) | Mean+ SD | RSD | Concentration (µg/ml) | Mean+ SD | RSD |
| 1. | 4 | 0.212±0.14 | 0.66 | 4 | 0.216±0.14 | 0.64 |
| 2. | 6 | 0.440±0.01 | 0.02 | 6 | 0.436±0.01 | 0.02 |
| 3. | 8 | 0.592±0.07 | 0.11 | 8 | 0.589±0.06 | 0.10 |
| 4. | 10 | 0.627±0.08 | 0.12 | 10 | 0.631±0.08 | 0.12 |

## CONCLUSION

A simple, precise, accurate, economic, sensitive, reliable and reproducible UV Spectroscopic method for estimation of Dolutegravir in pharmaceutical dosage form has been developed and validated. Hence the method can be easily and conveniently used for routine analysis of Dolutegravir in pure and its pharmaceutical formulations.

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