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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DOLUTEGRAVIR IN PURE FORM BY USING UV SPECTROPHOTOMETRY

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ABSTRACT

A simple, specific, accurate and precise spectroscopy method was developed and validated for the estimation of Dolutegravir in Pure form. The Standard solution was prepared by weighing 100 mg of Dolutegravir in 100 ml volumetric flask with dilute dimethyl formamide The final Standard solution was made to produce 1000 µg / ml with dilute dimethyl formamide Further dilutions were prepared as per procedure and were scanned at 260 nm. The linearity was found in the concentration range of 10-60 µg / ml. The Correlation coefficient was 0.999. The regression equation was found to be Y = 0.0552 X – 0.336. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and ruggedness,robustness. The limit of detection and limit of quantitation for estimation of Dolutegravir was found to be 0.09 (µg / ml) and 0.27 (µg / ml), respectively. The percentage recovery of Dolutegravir was found to be in the range of 98.49 ± 0.0001– 101.3 ± 0.003. Proposed method can be successfully applied for the quantitative determination of Dolutegravir in pharmaceutical Pure form.

INTRODUCTION

Analytical chemistry[1] is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present). Analytical chemistry is not a separate branch of chemistry, but simply theapplication of chemical knowledge.

Photometry including Photocolorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complexformation; acid - base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis.

Instrumental methods of chemical analysis: Instrumental method is an exciting and fascinatingpart of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences. Analytical instrumentation plays an important role in the production and evaluation of new products and in theprotection of consumers and environment.

Spectroscopy: Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted when the molecule or atoms or ions of a sample move from one energy state to another energy state. This change may be from ground state to excited state or excited stateto ground state.

Ultraviolet spectroscopy: Ultraviolet Spectroscopy is concerned with the study of absorption of UV radiation whichranges from 200 nm to 400 nm. Any molecule has n, π or σ combination of these electrons. Thesebonding (σ and π) and non bonding (n) electrons absorb the characteristic radiation and undergoestransition from ground state to excited state.

Visible Spectroscopy (colorimetry): Colorimetry is concerned with the study of absorption of visible radiation whose wavelength ranges from 400 nm to 800 nm.

Instrumentation

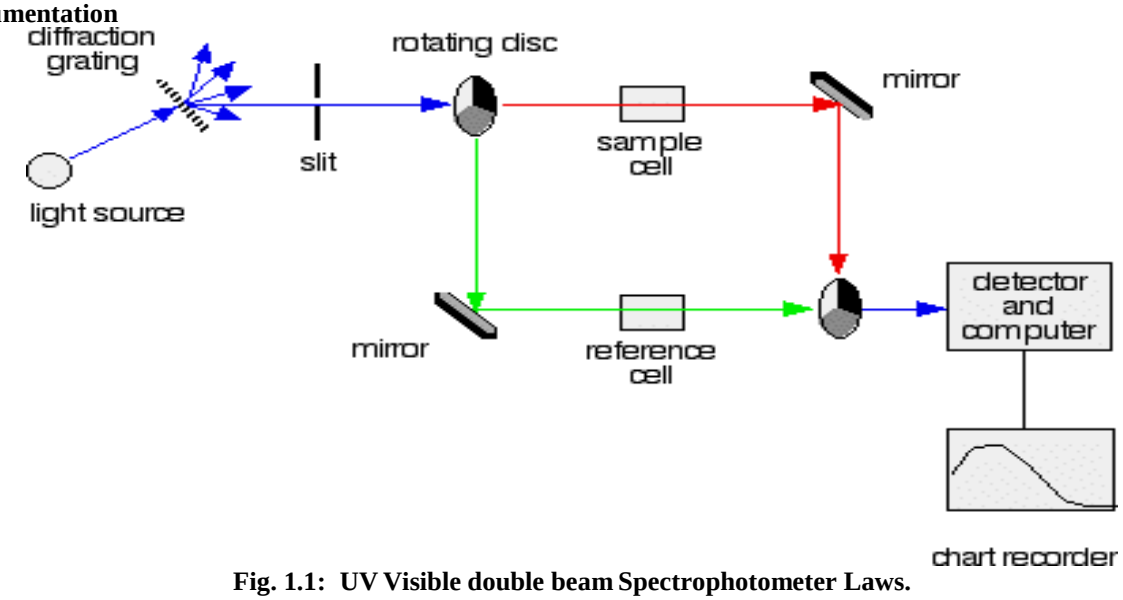


Fig. 1.1: UV Visible double beam Spectrophotometer Laws.

Governing Absorption of Radiation

Beer's law (related to concentration of absorbing species)

'The intensity of a beam of monochromatic light decreases exponentially with increase in theconcentration of absorbing species arithmetically'.

Lambert's law (related to thickness / path length of absorbing species)

'The rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light'.

Validation

Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

Types and level of validation

Full validation: This is important when developing and implementing a bio-analytical method for the first time. Full validation is important for a new drug entity. A full validation of the revised assay is important if metabolites are added to an existing assay for quantification.

Partial validation: These validations are modifications of already validated bio-analytical methods. Partial validation can range from as little as one intra-assay accuracy and precision determination to a nearly full validation.

Analytical method of validation

Method Validation can be defined as "Establishing documented evidence, which provides a High degree of assurance that a specific activitywill consistently produce a desired result or producea desired result or product meeting its determined specifications and quality characteristics".

Method validation study includes Accuracy, Precision, Linearity, Limit of detection, Limit of Quantitation, Robustness.

Linearity: A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) And /or separate weightings of synthetic mixtures of the drug product components, using the Proposed procedure.

Precision: Precision is the measure of how close the data values are to each other for a number of Measurements under the same analytical conditions. ICH has defined precision to contain three Components: repeatability, intermediate precision and reproducibility.

Accuracy: Accuracy is the measure of how close the experimental value is to the true value. Accuracy should be established across the specified range of the analytical procedure.

Specificity / Selectivity: The terms selectivity and specificity are often used interchangeably. According to ICH, the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other.

Limit of Quantitation: limit of quantitation is the lowest concentration of analyte is a sample that can Be determined with acceptable precision and accuracy under the stated experimental conditions. Several Approaches for determining the quantitation limit are possible, depending on whether the procedure is aNon-instrumental or instrumental.

Robustness: ICH defines robustness as a measure of the method capability to remain unaffected by Small, but

deliberate variations in method parameters.

Objectives

Drug analysis plays an important role in the development of drugs, their manufacture and therapeutic use. Pharmaceutical industries rely upon quantitative analysis to ensure that the raw material used and the final products obtained meet the required specifications.

A very few analytical methods appeared in the literature for the determination of Dolutegravir are generally based on HPLC, ultra violet spectroscopy (UV) and liquid chromatography – mass spectroscopy (LC-MS)), study on quantitative and qualitative analysis of Dolutegravir

In the present work, an attempt was made to provide newer, simple, accurate and low cost UV Spectroscopic method for the effective Quantitative estimation of Dolutegravir as an active pharmaceutical ingredient in pharmaceutical preparations without the interferences of otherconstituent in the formulation.

- Validation parameters
- Linearity
- Accuracy
- Precision
- Ruggedness
- Robustness
- Limit of Quantification
- Limit of detection.

All of the above mentioned methods were developed and validated statically to ensure their Accuracy, Precision, Linearity, Ruggedness and other analytical method validation parameters as mentioned in the various guidelines.

REVIEW OF LITERATURE

1. Thaidala Sriveni.et.al[12]

Pharmaceutical analysis plays a vital role in the quality assurance and quality control of bulk drugs. A simple, rapid, precise, and accurate spectrophotometric method has been developed for quantitative analysis of dolutegravir sodium in tablet formulations. The initial stock solution of dolutegravir sodium was prepared in methanol solvent, and subsequent dilution was done in water. The standard solution of dolutegravir sodium in water showed maximum absorption at wavelength 260 nm. The drug obeyed Beer–Lambert's law in the concentration range of 5–40 µg/mL with thecoefficient of correlation (R2) was 0.9992. The developed method can be adopted in routine analysis of dolutegravir sodium in bulk or tablet dosage form. The method can be used to determine the purity of the drug available from various sources.

2. K.Bhavyasri.et.al[13]

UV-spectrophotometric method for the determination of dolutegravir, an anti-retroviral drug, in bulk and pharmaceutical dosage form; and also to monitor the degradation behavior of the drug under different stress conditions according to ICH guidelines. 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 (r2) 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.

3. Sheeja.V.K.et.at[9,14]

A simple, rapid, accurate and economical method has been developed for the simultaneous estimation of Dolutegravir and Lamivudine in synthetic mixture by using the Q –absorbance ratiomethod. Absorbance ratio method for the ratio of absorbance at two selected wavelengths, one which is an iso - absorptive point and other being λ max of one of the two components. Dolutegravir and Lamivudine showed an absorptive point at 290 nm. The second wavelength used was 271 nm which is λ max of Lamivudine. The linearity of the method was found to be in the range of 1-5 µg /ml of Dolutegravir and 6- 30 µg /ml of Lamivudine. The concentration of the drugs was determined by using a ratio of absorbance at iso -absorptive point and the λmaxof Lamivudine.

4. Bhavar Girija Balasaheb.et.al[15]

A simple, rapid, precise and accurate spectrophotometric method has been developed for quantitative analysis of Dolutegravir sodium in tablet formulations. The initial stock solution of Dolutegravir sodium was prepared in methanol solvent and subsequent dilution was done in water. The standard solution of Dolutegravir sodium in water showed maximum absorption at wavelength 259.80 nm. The drug obeyed Beer–Lambert's law in the concentration range of 5–40µg/mL with coefficient of correlation (R2) was 0.9992. The method was validated as per the ICHguidelines. The developed method can be adopted in routine analysis of Dolutegravir sodium in bulk or tablet dosage form and it involves relatively low cost solvents and no complex extraction techniques.

5. Vaishnavi Dulange.et.al[16]

UV spectroscopic method was developed for the estimation of Dolutegravir in bulk and Formulation. The UV spectrum of Dolutegravir in methanol and water mixture showed λ max at 254nm. Beer's law is valid in the concentration range of 10-50µg/ml. This method was validated for linearity, accuracy, precision, LOD and LOQ. The method has demonstrated excellent linearity over the range of 10-50µg/ml with regression equation y = 0.030x + 0.008 and regression correlation coefficient r2= 0.998. Moreover, the method was found to be highly

sensitive with LOD (2.056µg/ml) and LOQ (6.230µg/ml). Depending on results the given method can be successfully applied for assay of Dolutegravir in formulation.

4. Drug Profile

Molecular Formula: $C_{20}H_{18}F_2N_3NaO_5$

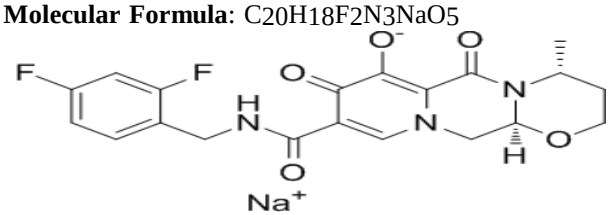


Fig. 4.1 structure of Dolutegravir.

Structure of Dolutegravir

Chemical Name: Sodium [4 R, 12aS]-9-[2,4-Difluorobenzyl (carbamoyl)]-4-methyl-6- 8- Dioxo-3,4,6,12,12a-Hexahydro-2H-Pyrido[1,2,4,5]Pyrazino[2,1-b] [1,3]Oxagin-7-olate

Category: HIV-Antiviral Agent

Molecular weight: 441.4g/mol

Melting Point: >300 **°C**

Solubility: ethanol, DMSO, Dimethyl formamide

Mechanism of action

Dolutegravir is an HIV-1 antiviral agent. It inhibits HIV integrase by binding to the active siteand blocking the strand transfer step of retroviral DNA integration in the host cell. The strandtransfer step is essential in the HIV replication cycle and results in the inhibition of viral activity.

Contraindications

- Autoimmune disease, Graves' disease, Guillain-Barre syndrome, immunereconstitution syndrome.
- Hepatic disease, hepatitis, Geriatric, Renal failure, Hepatitis C and HIV coinfection.

Uses

- Used to treat viral infections such as HIV.
- It is also used to treat cancer.

4. MATERIALS AND INSTRUMENTS

The following materials used were either AR/LR grade or the Possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Table 5.1: Materials.

S. No	Materials	Source
1	Dolutegravir	NATCO pharma Pvt.Ltd, HYD
2	DMF	FINAR CHEMICALS Pvt.Ltd AHMEDABAD
3	Water	GP CHEMICALS,KURNOOL

Table 5.2: Instruments.

S. No	Equipments	Source
1	UV Spectrophotometer	Analytical Technologies Limited 2080N
2	Sonicator	Wensar

6. Methodology

UV Spectroscopy

Analytical Technologies Limited UV-VIS 2080N Spectrophotometer was used with 1c m matched quartz cells. The data processing was performed using UV-probe software.

UV method development

The parameters for the development were as follows

1. Linearity
2. Accuracy
3. Precision
4. Robustness
5. Ruggedness
6. Limit of detection
7. Limit of quantification

Selection of wavelength

10mg/ml of Dolutegravir was scanned in the range of 200-400nm.

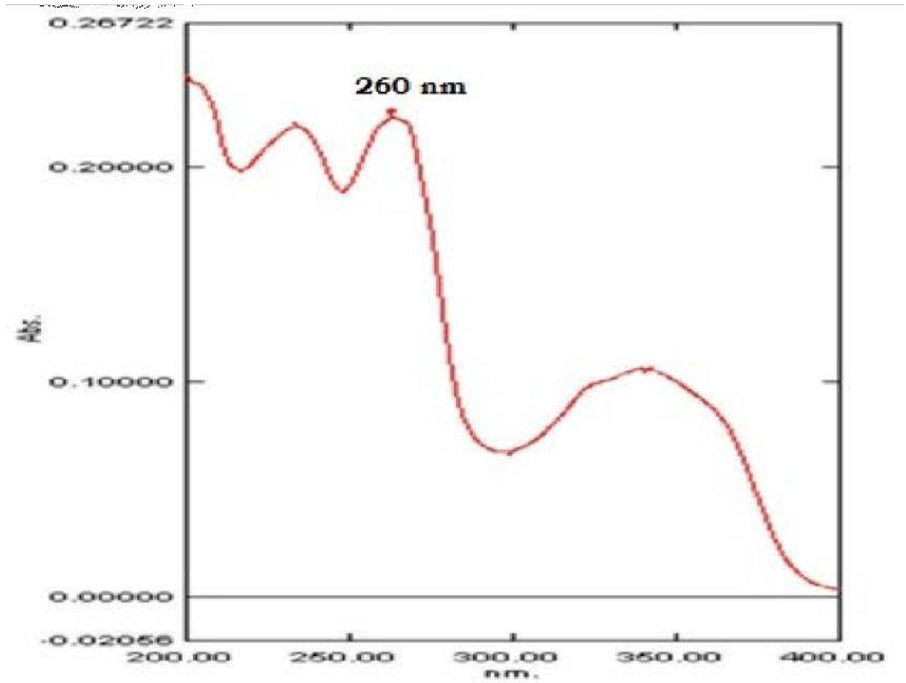


Fig. 6: I UV spectroscopy of Dolutegravir.

Validation of the Method
The method was validated in terms of parameters like linearity, accuracy, precision, LOD, LOQ, ruggedness, and robustness.

Preparation of dilute dimethyl formamide (DMF)
Take 5ml of Concentrated DMF and make upto to 100 ml with battery water.

Preparation of stock solution
100mg of Dolutegravir was dissolved in 5ml DMF in a 100 ml volumetric flask and solution was made upto volume with battery water upto 100 ml.

Preparation of standard working solution:
10ml of standard solution was dissolved in 100ml of volumetric flask and the solution was madeup to volume with battery water upto 100 ml.

Linearity
To evaluvate the linearity, serial dilution of analyte were prepared from the standard working solution was diluted with solvent to get a series of concentration ranging from 10,20,30,40,50 and 60 micro gram\ml. the prepared solution were filtered through whatman filter paper [NO.41]. Calibration curve was constructed by plotting the absorbance on y-axis against the concentration x-axis.

Precision
The precision of analysed method was studied by analysis of multiple sampling of homogeneoussample.

Intraday-Precision
In The Intraday Studies, the Standard Solutions (40mg/ml) Was Analysed for 6 Times in differenttimes.

Interval with in day. %RSD was Calculated presented in table 7.3

Inter day Precision
In the Inter-day variation studies, the standard solution (40mg/ml) was Analysed for 6 times n different days . %RSD was Calculated Presented In 7.4

Accuracy
Recovery studies by the standard addition method performed with a view to justify the accuracy of proposed method. previously analysed sample of Dolutegravir (45,55and 65microg/ml) were spiked with 80,100,120% extra Dolutegravir standard and the mixture were analysed by the proposed method. The experiment was performed in triplicate and recovery of thepure. %RSD was calculated and reported in table 7.9.

Sensitivity
The sensitivity of measuring of Dolutegravir by use of the proposed method was estimated in terms of limit od detection [LOD] and the limit of quantitation {LOQ}. The LOD and LOQ were calucated by the use of equation LOD=3.3 x σ/s and LOQ=10 x σ/s where σ is the standard deviation of response and S is the slope of the calibration curve LOD and LOQ values are reported in table7.10 and 7.11

1. Ruggedness
Ruggedness is the measure of the reproducibility of a test result under normal expected operating condition from instrument to instrument and analyst to analyst. The ruggedness of the method was determined by carrying out the experiment by different operations. The result of ruggedness testing is reported in the table 7.12

2. Robustness

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variation in the method condition, and is indication of the reliability of the method. A method is robustness, if it is unexpected by small changes in operating condition. To determine the robustness of this method, the experimental condition where deliberately altered at 3 different levels and responses were evaluated. Variation of wave length [258nm and 262nm] had no significant effect and the absorbance of 40 µg/ml Solution, indicating that the method was robustness. The result are shown in table 7.13 & 7.14.

3. RESULTS AND DISCUSSION

UV Spectroscopy Method

Table 7.1: Characteristic parameters of Dolutegravir for the proposed UVspectroscopy method.

Parameters	UV
Calibration range (µg/ml)	10-60(µg/ml)
Wavelength	260nm
Regression equation (y*)	0.0552x
Slope	0.0552
Correlation co efficient(r^2)	0.999
LOD (µg/ml)	0.09
LOQ (µg/ml)	0.27

Y*= bx+a where x is the concentration of Dolutegravir in µg/ml and Y is the absorbance at therespective ʎ max.

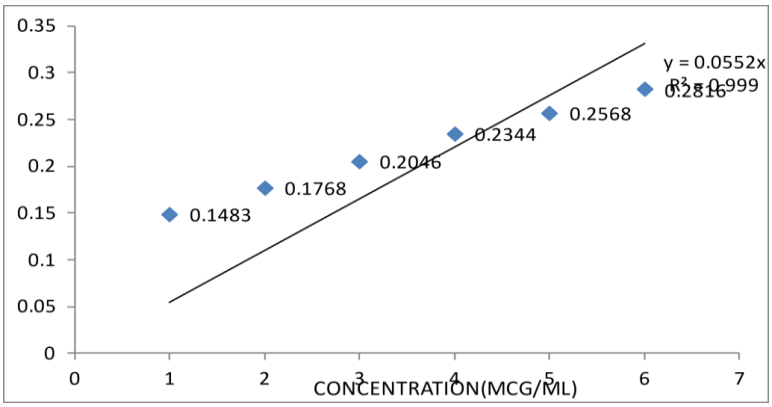
Validation of Analytical Method

***1.* Linearity**

Calibration graph were plotted using absorbance of standard drug versus concentration of standard drug solution. Linear regression data showed a good linear relationship over a Concentration range 10-60µg/ml.

Table 7.2: Calibration data of Dolutegravir.

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.1483
3	20	0.1768
4	30	0.2046
5	40	0.2344
6	50	0.2568
7	60	0.2816



Concentration(10µg/ml)

Fig. 7.1: Calibration curve of Dolutegravir.

Observation

1. The correlation coefficient for Dolutegravir was found to be 0.999 respectively.
2. The linearity range for Dolutegravir was found to be10-60µg/ml.

Precision

Table 7.3: Inter day precision.

0.S.No	Concµg/ml	Absorbance						AVG	SD	%RSD
		1	2	3	4	5	6			
1	40	0.2296	0.2304	0.2281	0.2296	0.2281	0.2304	0.2301	0.001539	0.6688
2	40	0.2304	0.2328	0.2304	0.2305	0.2328	0.2297	0.2310	0.001533	0.6636
3	40	0.2281	0.2297	0.2305	0.2328	0.2296	0.2305	0.2321	0.001544	0.6652
4	40	0.2297	0.2305	0.2296	0.2304	0.2297	0.2281	0.2317	0.001537	0.6633
5	40	0.2328	0.2281	0.2297	0.2281	0.2305	0.2296	0.2350	0.001542	0.6561
6.	40	0.2305	0.2296	0.2328	0.2297	0.2304	0.2328	0.2372	0.001558	0.6568

Acceptance criteria
%RSD of the six replicate injections should not more than 2.0%

Table 7.4: Intraday precision result.

S. No.	Concµg/ml	Absorbance						AVG	SD	%RSD
		1	2	3	4	5	6			
1	40	0.2308	0.2312	0.2308	0.2314	0.2356	0.2356	0.2306	0.0016	0.6956
2	40	0.2314	0.2356	0.2251	0.2312	0.2251	0.2308	0.2345	0.0021	0.8977
3	40	0.2251	0.2314	0.2356	0.2308	0.2312	0.2356	0.2367	0.0026	1.0984
4	40	0.2312	0.2356	0.2314	0.2356	0.2314	0.2251	0.2377	0.0030	1.2620
5	40	0.2356	0.2251	0.2312	0.2251	0.2308	0.2312	0.2354	0.0034	1.4443
6	40	0.2296	0.2308	0.2356	0.2356	0.2356	0.2314	0.2363	0.0036	1.5234

Acceptance criteria
%RSD of the six replicate injections should not more than 2.0%

2. Accuracy

Table 7.5: Observation for accuracy standard(50µg/ml).

S. No	Concentration(µg/ml)	Absorbance
1	Set-1	0.2127
2	Set-2	0.2073
3	Set-3	0.2098
4	**AVG**	0.2099
5	**SD**	0.002702
6	**%RSD**	1.28

Table 7.6: Observation for accuracy for 80% (45µg/ml).

S. No	Concentration(µg/ml)	Absorbance
1	Set-1	0.1886
2	Set-2	0.1876
3	Set-3	0.1893
4	**AVG**	0.1885
5	**Result**	44.90
6	**%Rec**	101.3
7	**SD**	0.00085
8	**%RSD**	0.453

Table 7.7: Observation for accuracy standard 100% (55µg/ml).

S. No	Concentration(µg/ml)	Absorbance
1	Set-1	0.2345
2	Set-2	0.234
3	Set-3	0.2335
4	**AVG**	0.234
5	**Result**	55.74
6	**%Rec**	98.49
7	**SD**	0.0005
8	**%RSD**	0.0117

Table No: 7.8.Observation for accuracy standard 120% (65µg/ml).

S .No	Concentration (µg/ml)	Absorbance
1	Set-1	0.2563
2	Set-2	0.2691
3	Set-3	0.2881
4	**AVG**	0.271167
5	**Result**	64.57
6	**%Rec**	99.8
7	**SD**	0.016
8	**%RSD**	0.405

Table 7.9: Accuracy Summary.

Sample (%)	Initial amount (µg/ ml)	Amount added (µg/ ml)	Amount recovered (µg/ ml)	%Recovery ±SD*	%RSD
80	40	5	44.90	101.3±0.003	0.453
100	50	5	55.74	98.49±0.000	0.011
120	60	5	64.57	99.8±0.016	0.405

*Average of three determinations

Acceptance criteria
1. %Recovery should be within the range of 98-102%
2. %RSD should be less than 2.

Sensitivity: Limit of detection of (LOD) and limit of quantitation (LOQ) were determined fromstandard and slope method as per ICH guidelines, for Dolutegravir LOD was found to be 0.09µg/ml and LOQ was found tobe 0.27µg/ml.

Table 7.10: Observation of LOD.

S. No.	Slope	SD of precision	LOD
1	0.0552	0.001539	0.09

Table 7.11: Observation of LOQ.

S. No.	Slope	SD of precision	LOQ
1	0.0552	0.001539	0.27

Ruggedness

Table 7.12: For Ruggedness (Analyst to Analyst).

S. No	Analyst-1		Analyst-2	
	Concentration(µg/ml)	Absorbance	Concentration(µg/ml)	Absorbance
1	40	0.2341	40	0.2268
2	40	0.2289	40	0.2289
3	40	0.2296	40	0.2312
	AVG	0.1929	**AVG**	0.2303
	SD	0.0022	**SD**	0.0026
	%RSD	1.1404	**%RSD**	1.1289

Acceptance criteria

%RSD of the six replicate injections should not more than 2.0%.

4. Robustness

Table 7.13: For 258and 262 wavelengths.

S. No	Concentration	Absorbance(at 258 nm)	Absorbance(at 262 nm)
1	Set-1	0.2105	0.2011
2	Set-2	0.2088	0.2114
3	Set-3	0.2111	0.2024
	AVG	0.2086	0.2064
	SD	0.0022	0.0023
	%RSD	1.0546	1.1143

Table 7.14: Robustness Summary.

S. No	Condition	Modification	Mean absorbance±S D*	%RSD forabsorbance
1	Wavelengt(nm)	230	0.2086±0.0022	1.0546
		234	0.2064±0.0023	1.1143

Average of the three Determination

Acceptance criteria

%RSD should not be more than 2.

DISCUSSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost UV-Visible Spectroscopic method. It is successfully applied for the determination of Dolutegravir pharmaceutical preparations without the interferences of other constituent in the formulations.

The optimum wavelength for detection was 260nm at which better detector response for the drug were obtained. The calibration was linear in concentration range of 10-60µg/ml in the Table7.2 for Dolutegravir respectively**.** The sensitivity for the drug has been calculated and the LOD and LOQ of the Dolutegravir was found to be 0.09 µg/ml and 0.27µg/ml in the Table 7.10 & Table 7.11.

The low values of % R.S.D. indicate the method is precise and accurate. The meanrecoveries were found in the range of 98-102% in the Table 7.9 for Dolutegravir respectively. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % R.S.D. reported was found to be less than 2 % in the Table 7.12.

Hence it is suggested that the proposed is UV / VIS Spectrophotometric method can be effectively applied for the routine analysis of Dolutegravir in bulk.

CONCLUSION

For routine analytical purpose it is always necessary to establish method capable of analysing huge number of samples in a short time period with due accuracy and precision.

Dolutegravir is not official in Pharmacopoeia. There is few analytical methods appeared in the literature for the determination of the Dolutegravir In literature review we have method only for the estimation of the above drugs of concern in individually or in combination of others.

In view of the above, a simple and specific analytical method was planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation of UV spectrophotometric method for the quantitative estimation of Dolutegravir in pure drug has been developed and validated.

The proposed UV method is more sensitive, accurate and precise and is suggestedfor routine analysis.

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