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Development of Analytical Method for *In-vitro* Drug Dissolution Study of Dolutegravir Marketed Formulation

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Abstract

Objective-The objective of this work is to develop and validate a dissolution test for dolutegravir tablet using a UV-Visible spectrophotometric method and high performance liquid chromatographic method.

Method-In UV-Visible spectrophotometric method, analysis of the drug was carried out on the 255nm wavelength and absorbance of dolutegravir sodium was determined by using phosphate buffer as solvent .while In the HPLC method, the analysis of drug was carried out on the Primesil C18 column (250 × 4.6 mm) 5 µm particle size using a mixture of methanol: water (pH 6) in the ratio of 70:30 % v/v as the mobile phase at the flow rate 0.8 mL/min at 255 nm at 40℃. This method was found to be linear in the concentration range of 5–35 µg /mL. The peak for dolutegravir sodium was observed at 5.8 ± 0.1 minutes.

Results-The methods were validated for linearity, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness, accuracy, and specificity. Release of more than 85% of the label amount was achieved over 8 hrs. In the medium throughout the study. The coefficient of correlation and percentage recoveries of dolutegravir sodium were 0.9999 and 99.9 %.Statistical analysis showed that both of the methods are repeatable and specific for the estimation of the said drug.

Conclusion-The proposed HPLC Method, UV-Visible spectrophotometric method, and dissolution test were validated as per ICH guidelines. The resulting method obtained was successfully applied for the quantitative determination of dolutegravir sodium tablet formulation. And it is also suitable for routine quality control analysis and in vitro dissolution studies.

Keywords: Dolutegravir, Dissolution method, HPLC Method, UV-Visible spectrophotometer, validation.

INTRODUCTION

In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality control purposes,i.e to asses batch to batch consistency of solid oral dosage forms such as tablet and drug development, i,e.,to predict in vivo drug release profile[1-2].

Dissolution is mainly defined as the process by which a substance forms a solution in a solvent. For the dissolution of solid, the process of dissolution can be explained as the breakdown of the crystal lattice into individual ions, atoms or molecules and their transport into the solvent. The primary goal of dissolution testing is to be used as a qualitative tool to provide measurements of the bioavailability of a drug and also to demonstrate the bioequivalence from batch-to-batch [3-6].

Dolutegravir (fig.1) is chemically known as : (4R,12aS)-N-[(2,4-diafluorophenyl)methyl]-7- hydroxy-4methyl-6,8-dioxo-3,4,12,12a-tetrahydro-2H-pyrido[5,6] pyrazino[2,6b] [1,3] oxazine-9-carboxamide [7].

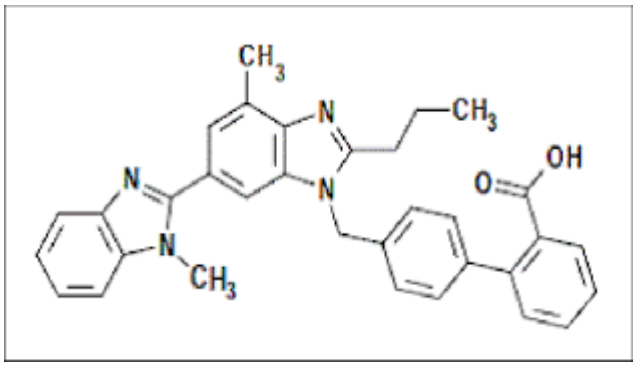

Fig.No : 1 Chemical Structure of Dolutegravir

Literature search revealed that as such there is a lack of method by which in vitro dissolution rate can be accurately quantified [8]. Although there are methods available for determination of dolutegravir for quantification of enantiomer [9], assay determination by HPLC [10], with different combinations [11], also the data of stabilizations are available [12].

The objective of this work is to develop and validate a dissolution test for dolutegravir tablet using a UV-Visible spectrophotometric method and high performance liquid chromatographic method.

This study, describes the development of a fast, accurate and precise HPLC method for determination of dolutegravir in pharmaceutical formulations and in dissolution media for drug quality control purposes. The dissolution method was also developed and validated according to USP guidelines [13].

MATERIAL AND METHOD

Chemical:

Dolutegravir drug was gifted from Lupin Pharmaceuticals Pvt Ltd Aurangabad, India, Disodium hydrogen phosphate (LR grade) Potassium dihydrogen phosphate (LR grade) Sodium lauryl sulphate (LR grade) Methanol (HPLC grade) ortho phosphoric acid (AR grade) water (HPLC grade).

Equipment:

1. DISSOLUTION INSTRUMENTS–Make–Electrolab technology, Apparatus-USP, Type II (Paddle)
2. UV-VISIBLE SPECTROPHOTOMETER -Make-Agilent, Software-carry win UV 60
3. HPLC.Make-Agilent, Software-EZ chrome Elite
5. PH METER.Make-Hanna instruments
6. ANALYTICAL BALANCE. Make-Winsor india
7. ULTRA SONICATOR.Make-Winsor, WUC Series
8. FTIR-spectrophotometer Make- perkin elmer, FTIR-ATIR, Spectrum two

Procedure:

Preparation of phosphate buffer pH 6.8:

28.80 g of disodium hydrogen phosphate and 11.45 g of potassium dihydrogen phosphate was accurately weighed and transferred in1000ml volumetric flask, to its approximately 50 ml of distilled water was added to dissolve the content and then final volume was then made up to the mark with water.

Preparation of standard stock solution:

Standard stock solution of dolutegravir was prepared by dissolving 10 mg of drug separately in 10mL volumetric flask using pH 6.8 phosphate buffer as solvent. Stock solutions of 1000 µg/mL were obtained in this manner. From these stock solutions, working standard solutions of concentration 100 µg/mL each were prepared by appropriate dilutions. Working standard solutions were scanned in the entire UV range to determine the λmax. The λmax of dolutegravir were found to be 255 nm. The maximum absorption (λmax) of Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths.

Calibration curves:

Seven standard dilutions of each drug were prepared separately having concentrations of 5-25 µg/mL. The absorbance of these standard solutions were measured at 255 nm calibration curve was plotted. The absorptivity coefficients of the drugs were determined using calibration curve.

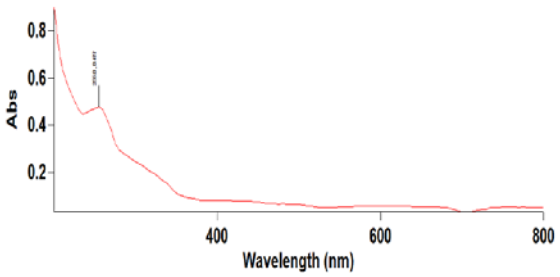

Fig No : 2 Wavelength Scan of Dolutegravir.

***In-vitro* Dissolution test:**

The drug release profile of dolutegravir tablets was determined in 900ml buffer solutions of pH 6.8 plus 0.25% SLS prepared according to USP at 50 rpm in apparatus II at temperature 37 °C±0.5.10ml of samples were collected after predetermined time interval i.e., 1hr, 2hr, 4hr, 6hr and 8hr and replaced by freshly prepared medium at the same time in order to maintain sink condition. Further dilution of samples is made by using 5, 10, 15,20,25,30 & 35 ppm with respective medium. Absorbance of all the samples is taken at wavelength of 255 nm by UV Spectrophotometer so that concentration in each time interval can be determined.

Chromatographic method:

An HPLC method with UV detection was selected for the method of analysis. The procedure utilized a column, C18, 250 mm x 4.6 mm, 5µ and UV detection at 255 nm. This wavelength was selected because it is a UV maximum and provides enough sensitivity needed for quantitation of this drug concentration in the dissolution samples. The column temperature was maintained at 35°C. The mobile phase contained methanol and water (pH 6) (70:30). The flow rate was 0.8 mL/ min with an injection volume of 20 µL. A standard solution of active pharmaceutical ingredient (API) was prepared first in mobile phase, and subsequently diluted down to the appropriate concentration with dissolution medium [14, 15].

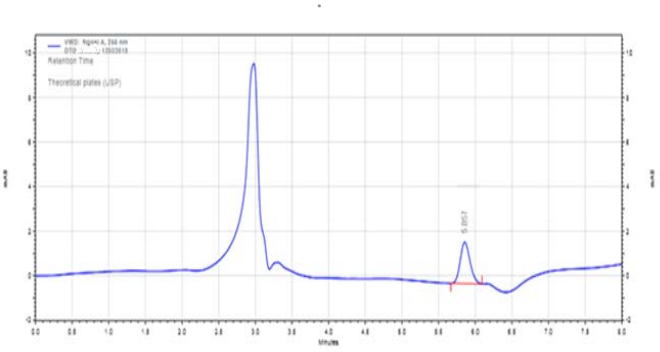

Fig No : 3 Standard chromatogram of dolutegravir on HPLC.

Statistical Analysis:

Descriptive statistics (mean±SD, and Percent Release) provided for measuring demographic variables. The dissolution test was performed and % release was determined. All the analysed data p < 0.05 was considered to be statistically significant.

RESULTS & DISCUSSION

Specificity:

An HPLC method with UV detection was selected for the method of analysis. The procedure utilized a column, C18, 250mm x 4.6 mm, 5µ and UV detection at 255 nm. This wavelength was selected because it is a UV maximum and provides enough sensitivity needed for quantitation of this drug concentration in the dissolution samples. The column temperature was maintained at 35°C. The mobile phase contained methanol and water (pH 6) (70:30). The flow rate was 0.8 mL/ min with an injection volume of 20 µL. A standard solution of active pharmaceutical ingredient (API) was prepared first in mobile phase, and subsequently diluted down to the appropriate concentration with dissolution medium.

System suitability testing:

System suitability is a pharmacopoeia requirement and is used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The test were performed by collecting data from six replicate injection of standard drug solution [17].

Linearity:

Linearity of the dolutegravir of the dissolution test method was demonstrated in the range of 5-30 ppm (5, 10,15,20,25 and 30 ppm) of target concentration of analyte. Prepared solutions were injected in duplicate and linearity graphs of concentration in ppm (X-axis) versus absorbance (Y-axis) were plotted.

Correlation coefficient, square of correlation coefficient, slope of regression, relative standard deviation of response factor were calculated for analyte peak. The correlation coefficient was found to be 0.9999 for dolutegravir which was far better than the acceptance criteria of 0.9900.

Table.No :1 Linearity of dolutegravir by UV-Visible spectrophotometer.

Sr.no	Concentration (ppm)	Absorbance (nm)
1	5	0.1002
2	10	0.1914
3	15	0.2847
4	20	0.3845
5	25	0.4754
6	30	0.5698
	Slope	0.0474
	Intercept	0.0023
	correl	0.9999

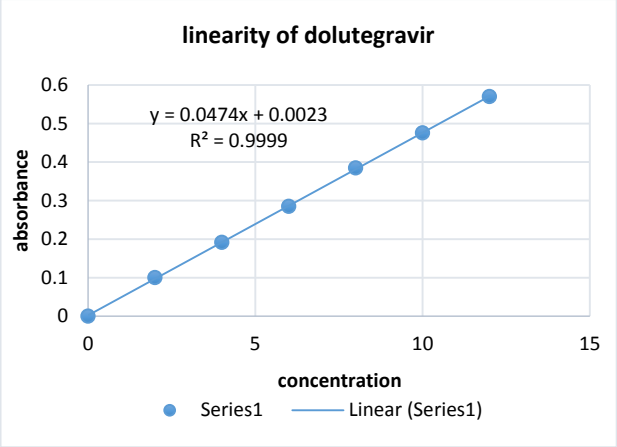

Fig No : 4 Linearity graph for Dolutegravir on UV-Visible spectrophotometer.

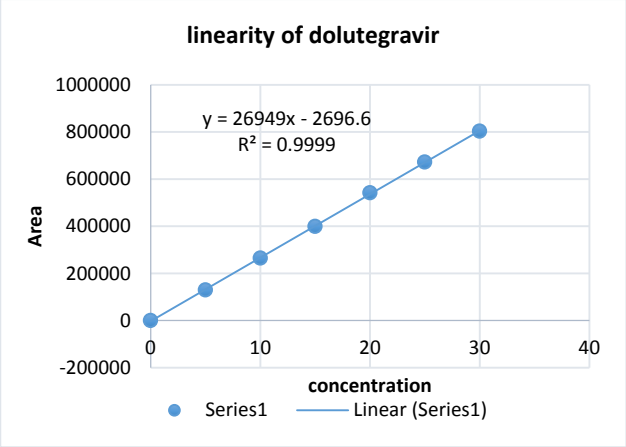

Fig No : 5 Linearity graph for dolutegravir on HPLC.

Table. No : 2 Linearity of dolutegravir by HPLC.

Sr.no	Concentration (ppm)	Peak area
1	5	129608
2	10	264511
3	15	398920
4	20	522081
5	25	672411
6	30	803220
	Slope	2696.6
	Intercept	26949
	correl	0.9999

Accuracy (by recovery):

Accuracy of test method was performed in the range of 80.0%, 100.0% 120.0%) of target concentration of analyte. Triplicate sets of samples at each concentration were prepared and injected by single injection into the liquid chromatography system and chromatograms were recorded. The two acceptance criteria were placed for conformance of accuracy, the % RSD of all the sets shall not exceed 2.0% and the % recovery shall be within 95.0% to 105.0% for all the sets **(Table 3).**

Precision:

Preparation of standard stock solution:

A standard 100 ppm stock solution was prepared by transferring 61.6 mg of working standard (equivalent to about 50mg dolutegravir) to a 50ml of volumetric flask and sonicated for 5 to 10 min and make up the remaining volume with phosphate buffer.

Table. No : 3 Comparision in Accuracy of dolutegravir by UV-Visible spectrophotometer and HPLC.

Sample Identity	Actual amount added in mg	UV-Visible spectrophotometer			HPLC		
		Absorbance (nm)	Amount recover in mg	Amount recover in %	Peak area	Amount recover in mg	Amount recover in %
80 %	18	0.5378	18.02	100.1	475887	17.99	99.95 %
80 %	18	0.5402	18.10	100.5	476868	18.02	100.1 %
80 %	18	0.5368	17.99	99.97	476794	18.02	100.1 %
100 %	20	0.5969	20.01	100	528898	19.99	99.97 %
100 %	20	0.5970	20.01	100	531147	20.08	100.4 %
100 %	20	0.5965	19.99	99.98	529098	20	100 %
120 %	22	0.6560	21.99	99.96	582012	22	99.99 %
120 %	22	0.6570	22.02	100.1	582224	22.01	99.98 %
120 %	22	0.6568	22.01	100	582414	22.01	100 %
			Mean± SD	100.06± 0.17		**Mean±SD**	100.05±0.14
			%RSD	0.17		**% RSD**	0.139

The result represent in mean±SD, n =6.

Table. No : 4 Comparison in precision of dolutegravir by UV-Visible spectrophotometer and HPLC.

Sr.No	UV-Visible spectrophotometer				HPLC			
	Concentration (ppm)	System precision absorbance (nm)	Method precision absorbance (nm)	% of release	Concentration (ppm)	System precision Peak area(nm)	Method precision Peak area(nm)	% of Release
1	10	0.6699	0.4851	100.1	10	442085	433520	99.97
2	10	0.6647	0.4798	99.08	10	442156	433422	100
3	10	0.6714	0.4848	100.1	10	442250	429999	100
4	10	0.6687	0.4850	100.1	10	445136	433224	99.98
5	10	0.6741	0.4858	100.3	10	445562	429885	99.99
6	10	0.6678	0.4799	99.10	10	445148	430012	100
	Mean±SD	0.6694±0.0032	**Mean±SD**	99.79±0.55	**Mean±SD**	443722±1715.656	**Mean±SD**	99.99±0.012
	%RSD	0.47	**%RSD**	0.55	**%RSD**	0.38	**% RSD**	0.01

The result represent in mean±SD, n =6.

Table. No : 5 Comparison in Ruggedness of Dolutegravir by UV-Visible spectrophotometer and HPLC.

Sample identity	UV-Visible spectrophotometer				HPLC			
	Tablet wt. in mg	Concentration (ppm)	Absorbance (nm)	Amount recover (%)	Tablet wt. in mg	Concentration (ppm)	Peak area	Amount recover (%)
Ruggedness-1	308.12	10	0.4788	98.87	308.12	10	429091	99.92
Ruggedness-2	308.12	10	0.4790	98.91	308.09	10	429298	99.97
Ruggedness-3	308.12	10	0.4840	99.94	308.07	10	432712	100.7
Ruggedness-4	308.12	10	0.4841	99.96	308.1	10	431014	100.3
Ruggedness-5	308.12	10	0.4843	100	308.11	10	429290	99.97
Ruggedness-6	308.12	10	0.4839	99.92	308.4	10	429294	99.98
			Mean±SD	99.60±0.55			**Mean±SD**	100.1±0.30
			% RSD	0.55			**% RSD**	0.30

The result represent in mean±SD, n =6.

System Precision:

Five replicate of standard solution at working concentration were analysed and the absorbance was recorded.

Method Precision:

Six tablets were weighed. One tablet was placed in each of the six respective dissolution vessels and dissolution test was started. At the specified time points of 8 hrs. 10 mL of aliquot was withdrawn from each dissolution vessel. The withdrawn solutions was then filtered through 0.45 µm nylon filter and dilute this solution from 1ml to 10ml and absorbance was measured. % Release for each analysis was calculated.

Ruggedness (intermediate precision):

It is the degree of the reproducibility of the test result obtained by analysis of samples, under a variety of condition such a different lab, analyst, instruments, lots of reagents, elapsed time, different time, temp, etc.

Determination:

The ruggedness of an analytical method is determined by analysis of aliquots from homogeneous lots by different analyst using operational and environmental condition that may differ but still within the specified parameter of the assay. The degree of the reproducibility of test results is then determined as a function of the assay variables.

10mlof aliquots are withdraw from vessel and transferred from 0.45 µm nylon filter. After filtration the 6replicate sample was prepared from that aliquots. And analysed on UV-Visible spectrophotometer and HPLC.

Table. No : 6 Comparison of Limit of Detection and Limit of Quantitation by UV-Visible spectrophotometer and HPLC.

Sr.No	Parameter	UV-Visible spectrophotometer	HPLC
1	Limit of detection	1.34 ppm	0.85 ppm
2	Limit of quantitation	4.07 ppm	2.60 ppm

DISCUSSION

The results obtained in the preliminary studies were satisfactory. After setting dissolution parameters, the UV and chromatographic parameters were optimized. The release pattern of dolutegravir is about 85% as compared to other it shows better sustained release pattern. The result obtained by UV & HPLC was satisfied [18]. After optimizing all the parameters, the method was checked for quality control purpose successfully.

ABBREVIATIONS

UV-Ultra Violet , HPLC-High performance liquid chromatography , API –Active pharmaceutical ingredients, RPM-Revolution per minutes , %-percent , ppm-part per millions , nm-Nano meter , wt.-weight , temp-temperature , hrs.-hours , SD-standard deviation , RSD- Relative standard deviation µm-micro meter , µL-micro litre , mg-micro gram , mL-mili litre.

CONCLUSION

The method has been shown to be linear, precise and accurate across a suitable analytical range. Solutions have been shown to be stable for at least 24 hours at room temperature storage condition. The method shows that there is negligible effect of filtration so sample required to saturate the membrane filter of 0.45µ is 5ml. The method is shown to be robust for the small, deliberate changes made in conditions. So we finalized Apparatus as USP Type 2 (paddle), Medium as pH 6.8 phosphate buffer, RPM as 50 and single time point at 8hrs.

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AUTHOR CONTRIBUTION

Study conception and design: Dr.Sushil P.Narkhede, Mrs.Madhavi P.Shinde.
Acquisition of data: Ujjwala A.Mulak.
Analysis and interpretation of data: Ujjwala A.Mulak.
Drafting of manuscript: Ujjwala A.Mulak.

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