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Novel RP-HPLC Method Development and Validation for Estimation of Dolutegravir in Bulk Dosage Form

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ABSTRACT:

A rapid and accurate method has been developed to evaluate dolutegravir dosage forms by RP HPLC. The results indicate that the proposed method has high sensitivity and accuracy to measure the API content in commercial formulations of dolutegravir has been successfully performed. Detection of dolutegravir was performed at 254 nm using a Waters SunfireC18 column 150 mm 4.6 mm and a mobile phase consisting of pH 3.0 phosphate buffers and methanol (70:30), at flow rate 0.3 and retention time of standard at 3.5min and total run time of the analysis is 8mins. The linear range of dolutegravir was determined to be between 50µg/mL and 150 µg/ ml R2 0.999, Regression equation of dolutegravir is y = 21519x+85441 and the %RSD of dolutegravir found was 1.0. the amount of dolutegravir showed a recovery rate of 99.4%. Retention time was decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Dolutegravir, HPLC, Method development and validation, ICH guidelines

1. Introduction:

AIDS is considered as the final chapter in the life of HIV infected patients. Pre-exposure prophylaxis (PrEP) with oral antiretroviral medicines has recently been shown to minimise the risk of HIV infection. To prevent the repeated dose and to achieve better efficacy generally fixed-dose combinations are used. In HIV therapy, DTG is used as the sodium salt in combination with two reverse transcriptase inhibitors typically lamivudine and tenofovir disoproxil or tenofovir alafenamide, or lamivudine and abacavir . Dolutegravir(DTG) is a second-generation drug,The drug acts via chelation of the two magnesium ions in the active site of the HIV reverse transcriptase and a hydrophobic interaction of the fluorinated phenyl ring within a pocket . It works by blocking integrase and prevents HIV from Replicating and lowers the amount of HIV in the blood. Dolutegravir sodium, chemically known as (4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido [1',2':4,5] pyrazino[2,1-b][1,3] oxazine-9-carboxamide. USFDA approved three drugs combination therapy (abacavir 600 mg, lamivudine 300 mg, dolutegravir 50 mg) as single pill regimen (combined dosage form) for people living with HIV. Literature survey revealed that there were few RP-HPLC methods reported for Dolutegravir (13-20). An extensive literature search revealed the retention times are long for Dolutegravir in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the simultaneous estimation of Dolutegravir in API and Pharmaceutical dosage form.

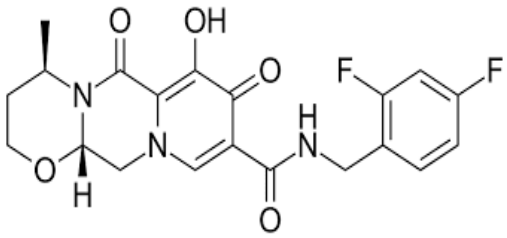

Figure.1. Structure of Dolutegravir

2. Materials and Methods:

2.1. Materials for Experiment

2.1.1. Standard: Dolutegravir **(**Gift samples obtained from Madras pharmaceuticals, Chennai**)**

2.1.2. Sample: INSTGRA 50mg tab (Obtained from local pharmacy)

2.1.3. Chemicals and Reagents: HPLC grade solevnts like Orthophosphoric acid, Methanol, Acetonitrile are purchased from Rankem, AR grade reagents like Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride are purchased from Merck and Ultrapure water form Rephile.

2.2. Instruments used:

Electronic balance make Shimadzu, Syringe made Hamilton,Ultra sonicator make by Citizen, Digital Ultrasonic Cleaner, pH meter make by Global digital, UV-Visible Spectrophotometer made by Nicolet evolution 100 with operating software Vision Pro, HPLC made by Shimadzu(LC 20 AT VP) with operating software Spin chrome (LC SOLUTIONS) ,HPLC column sunfire C18 (150x4.6mm) 3.5um.

2.3. Preparation of stock solutions:

2.3.1.Diluted Orthophosphoric Acid Preparation procedure: dissolve 5 ml of orthophosphoric acid (85%) in distilled water up to 100 ml and mix well.

2.3.2.Preparation of 0.1 molar sodium hydroxide: dissolve the 0.4g sodium hydroxide in 100 mL of unadulterated water and blend well.

2.3.3. Preparation of sodium chloride solution: Add 4.5 grams of sodium chloride to 500 ml of freshly filtered water and stir to dissolve.

2.3.4. Procedure for standard stock solution Preparation (About 1000 ppm): 100 mg of Dolutegravir was weighed and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and label as 1000 µg/mL solution.

2.3.5. Procedure for Dolutegravir Working standard solution preparation (about 100ppm): Pipette out 1 mL volume solution from Dolutegravir standard stock preparation into 10 mL volumetric flask. Add the required amount Diluent up to mark and mix well and label as 100 µg/mL solution.

2.3.6. preparation Sample stock solution for Assay:, 100 mg of Dolutegravir (INSTGRA 50mg tab) was weighed and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 100 µg /ml of solution by diluting 5ml to 50ml with mobile phase.

2.3.7. Preparation of Sample working solution:

Weigh a quantity of powder equivalent to 100mg of Dolutegravir and transferred in to 100 ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 100 µg /ml of solution by diluting 1ml to 10ml with mobile phase.

3. Method Development for Dolutegravir

3.1. Solubility Studies:

10 mg of Dolutegravir was weighed and transferred in to three different 100 ml volumetric flask and dissolved in water, methanol, ethanol and then make up to the mark with solvents and prepare 100 µg /ml of solution by diluting 1ml to 10ml with solvents.

Table 1. Solubility studies.

Solvent Name	**Dolutegravir**
Water	Sparingly Soluble
Methanol	Soluble
Ethanol	Soluble

Observation: Methanol is selected for initial standard stock solution preparation

3.2. Determination of Working Wavelength (λ_{max})

Preparation of Standard solution 10 mg of Dolutegravir was weighed and transferred in to 100 ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 100 µg /ml of solution by diluting 1ml to 10ml with methanol.

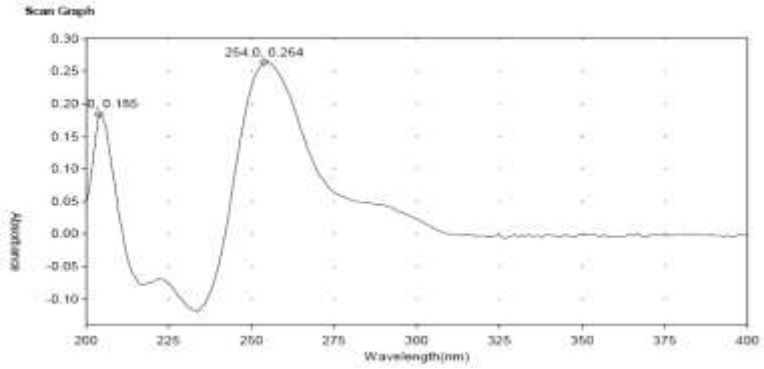

Figure. 2.UV-VIS Spectrum of Dolutegravir (254 nm)

Observation: The wavelength of maximum absorption (λ_{max}) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 254nm for Dolutegravir (Fig.2), 254 nm was selected as detector wavelength for the HPLC chromatographic method.

3.3. Optimization of Chromatographic conditions:

The separation was performed on Waters Sun fire C18 (150×4.6 ×3.5µ) using

Mobile phase pH 3.0 Phosphate Buffer: Methanol (70:30). In simple gradient mode at a flow rate of 1.0mL/min and injection volume of sample was 10 µL. The column temperature was maintained at 25°C and samples were analyzed at 254 nm detector wavelength. The retention time was 3.107min with total run time of 8 min.

4. Results and Discussion:

Method validation: Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

4.1. System Suitability:

System suitability is an integral part of chromatographic system. The calculation and comparison of verified resolution, capacity factor, tailing factor, theoretical plate count with standard specification of system. The column was equilibrated with mobile phase for 30min with flowrate 1.5mL/min and dolutegravir standard with 100 ppm concentration was injected sixes times into HPLC system after the injecting of one blank.

Table .2.Results for system suitability of dolutegravir.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	3.107	2073724	4578	1.2
2	3.108	2074581	4582	1.1
3	3.105	2074142	4576	1.2
4	3.102	2073614	4852	1.0
5	3.105	2073965	4563	1.1
6	3.106	2073654	4577	1.3
Mean	3.106	2073947	-	-
SD	0.00	370.613365	-	-
%RSD	0.1	0.0	-	-

Discussions: The plate count and tailing factor of dolutegravir results were found to be within the limits and the % RSD was found to be 0.1 so system is suitable and giving precise results.

4.2. Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Blank solution was injected and the chromatogram was recorded for the same. Placebo solution was prepared and it was injected and the chromatogram was recorded for the same.

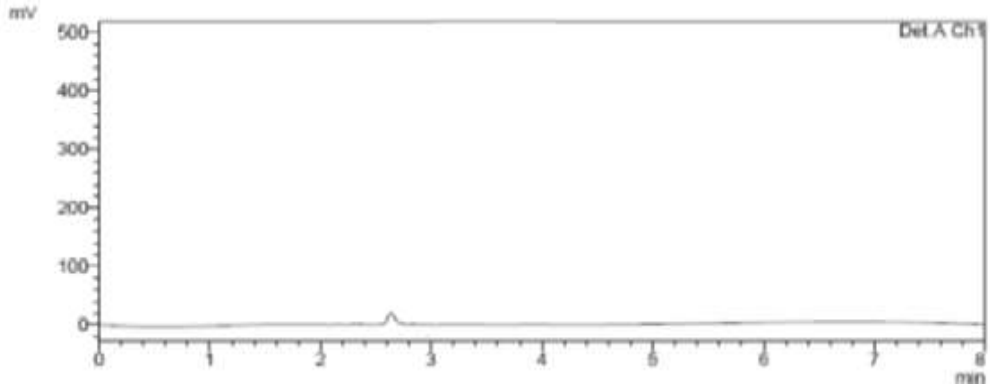

Figure.3. Chromatogram of Placebo

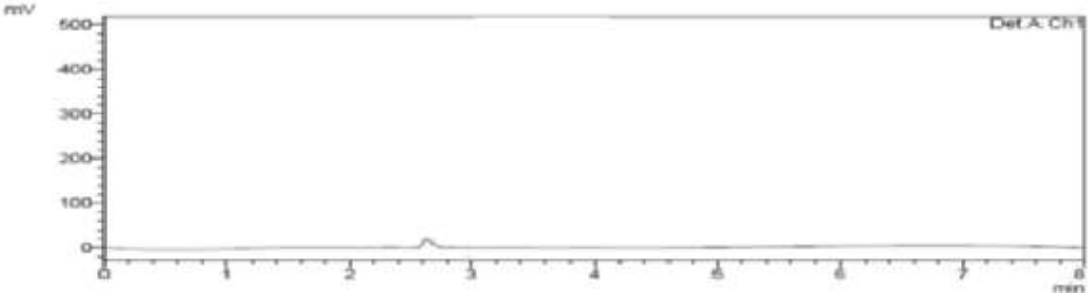

Figure.4. Chromatogram of Blank

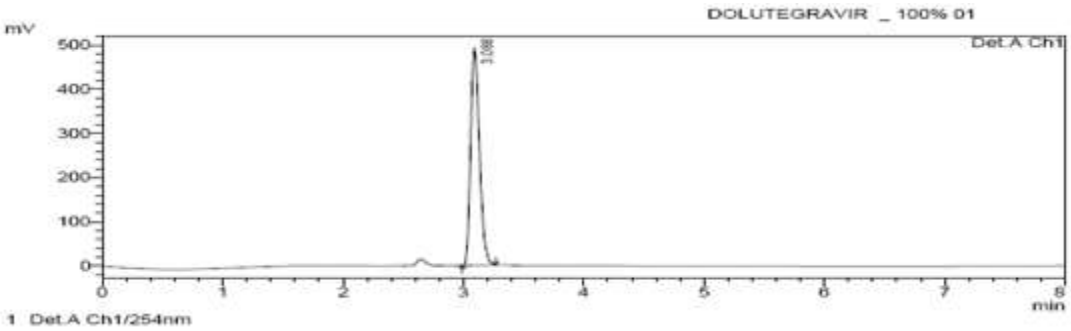

Figure.5.Chromatogram of Dolutegavir Standard 100&%

Discussions: Chromatograms of blank and placebo solutions had shown no peaks at the retention times of Dolutegravir. It was observed that diluent or excipient peaks do not interfere with the Dolutegravir Peak.

4.3. Method precision:

Method precision was determined by injecting sample solutions of concentration DOLUTEGRAVIR (10µg/mL) for six timesare prepared separately

Table .3. Method precision results for Dolutegravir

DOLUTEGRAVIR		
S. No	**Area**	**%Assay**
1	2073796	101.3
2	2036834	98.5
3	2078955	100.8
4	2075109	100.1
5	2063159	100.8
6	2075519	100.6
Avg		98.7
Std dev		1.01
%RSD		1.0

Discussions: The %RSD of Assay for 6 Samples determinations of DOLUTEGRAVIR found to be within the acceptance criteria (less than 2.0%). Hence method is precise.

4.4. Linearity and range:

Linearity of an analytical method is carried out to demonstrate that concentration of an analyte is directly proportional to the peak area of analyte. The evaluation of linearity is done by visual inspection of plot of signal as a function of analyte concentration in sample. The linearity was determined by analysing six solutions over the concentration range of 50-150 µg/mL.

Table .4. Linearity data of dolutegravir

S. No	Concentration (µg/mL)	Area
1	50	1170177
2	75	1697912
3	100	2207163
4	125	2804924
5	150	3306552
	Correlation coefficient	0.9993
	Slope	21519
	Intercept	85441

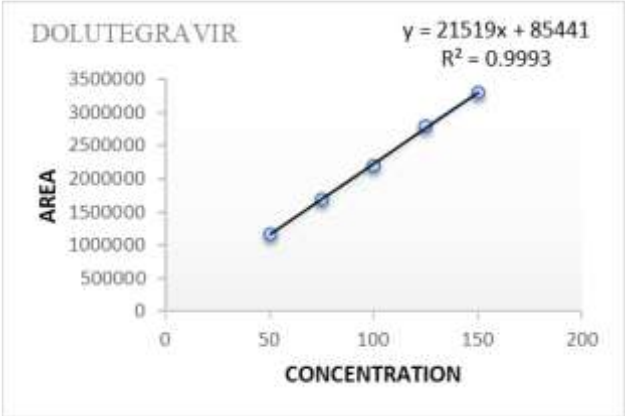

Figure.6. Linearity curve of Dolutegravir

Discussions: The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparation was found to be 0.9993.regression coefficient was found intercept is 21519 and slope is 85441.

4.5. Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs **(50µg/ml, 100µg/ml and 150µg/ml)** were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

Table.5. Results for Recovery of Dolutegravir

DOLUTEGRAVIR						
Name of the Sample	**Standard Weight in mg**	**Area**	**Conc Added (µg/ml)**	**Conc Recovered (µg/ml)**	**%Recovery**	**Average**
50% Recovery_01	50	1000541	50	49.35	98.7	99.4
50% Recovery_02	50	1005847	50	49.61	99.2	
50% Recovery_03	50	1000584	50	49.36	98.7	
100% Recovery_01	100	2031012	100	100.18	100.2	
100% Recovery_02	100	2021854	100	99.73	99.7	
100% Recovery_03	100	2002541	100	98.78	98.8	
150% Recovery_01	150	3032514	150	149.58	99.7	
150% Recovery_02	150	3014251	150	148.68	99.1	
150% Recovery_03	150	3047412	150	150.32	100.2	

Discussion: The percentage mean recovery of Dolutegravir was found between 99.0 to 102.0

4.6. Limit of Detection:

From the standard stock solution was 4ml pipetted out and transferred to separate 10ml flask and made up with diluent. From above solutions 0.1ml of Dolutegravir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

$LOD = \frac{3.3\sigma}{S}$ = 3.3 * (21519)/25245 = 3.87µg/ml (DOLUTEGRAVIR)

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Discussion: The LOD for this method was found to be 3.87µg/ml (DOLUTEGRAVIR)

4.7. Limit of Quantification (LOQ):

From the standard stock solution was 4ml pipetted out and transferred to separate 10ml flask and made up with diluent. From above solutions 0.3ml of Dolutegravir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

$$LOQ = \frac{10\sigma}{S} \qquad = 10 * (21519)/25245 \qquad = 11.61.\mu g/ml \text{ (DOLUTEGRAVIR)}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Discussion: The LOQ for this method was found to be 11.61µg/ml (DOLUTEGRAVIR)

4.8. Robustness:

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table .6.

Table.6. Results for Robustness of Dolutegravir

Chromatographic changes		**Retention time(min)**	**Tailing Factor**	**Theoretical Plates**
Flow rate (mL/min)	1.0	3.671	1.338	53783
	1.2	3.084	1.348	46014
wavelength (nm)	252	3.081	1.352	46084
	256	3.086	1.352	46799

Discussion: The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

4.9. Ruggedness:

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Table.7. Ruggedness Results of DOLUTEGRAVIR

DOLUTEGRAVIR	**%Assay**	**DOLUTEGRAVIR**	**%Assay**
Analyst 01	99.92%	**Analyst 01**	98.64%
Analyst 02	98.36%	**Analyst 02**	99.60%
%RSD	0.11	%RSD	0.28

Discussion: From the above results % Assay and %RSD obtained acceptance criteria 2% so method is rugged.

5.Conclusion:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dolutegravir in bulk and dosage form. Retention time of Dolutegravir was found to be 3.107min. %RSD of the Dolutegravir was found to be 1.0. %Recovery was obtained as 99.4% for Dolutegravir. LOD, LOQ values obtained from regression equations of Dolutegravir was 3.87µg/ml, 11.61µg/ml. Regression equation of Dolutegravir is y = 21519x + 85441. Retention time was decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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1. Dr. M. Ajitha, Head of the department, Centre for Pharmaceutical Sciences, UCEST, JNTUH Hyderabad.

2. Dr. M. Sunitha Reddy, Professor and Principal, UCPSC, Sulthanpur, JNTUH, Hyderabad, T.S

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