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DEVELOPMENT AND VALIDATION OF RP-HPLC Method For Estimation Of Antiretroviral Drug In Tablet Dosage Form By Quality By Design Approach (Drug -Dolutegravir)

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ABSTRACT
Dolutegravir is the newest integrase strand transfer inhibitor to be approved for the treatment of human immunodeficiency virus (HIV) infection. Dolutegravir is equivalent or superior to existing treatment regimens in both treatment-naïve and treatment-experienced patients including those with previous raltegravir or elvitegravir failure. The consistent efficacy coupled with excellent tolerability and infrequent drug–drug interactions make the co-formulation of dolutegravir with two nucleotide reverse-transcriptase inhibitors an attractive treatment option. This review summarizes the pharmacokinetics, adverse event profile, and efficacy of dolutegravir in the treatment of HIV.
Dolutegravir can cause serious, life-threatening side effects. These include severe skin rash and allergic reactions, liver problems, and drug interactions. Contact your health care provider right away if you develop a rash while taking dolutegravir.
KEY WORDS: Dolutegravir, Raltegravir, Elvitegravir

I. INTRODUCTION

Quality assurance is a wide-ranging concept covering all matters that individually or collectively influence that quality of the product. It plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key role to the design, development, standard and quality control of medicinal product.

Quality of the drug product is very vital, as it involves life. Proper manufacture and quality control of pharmaceuticals is the vital segment of strong primary healthcare programme worldwide. Quality is the total sum of all factors which contribute directly or indirectly to the safety, efficacy and acceptability of the product.

Pharmaceutical analysis, a branch of pharmacy, plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulation and on finished products. Analytical chemistry has since long, occupied an important place in the development of science and technology. It is primarily concerned about determining the qualitative and quantitative composition of material under study. The qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. The quantitative analysis deals about the content present in the sample. The development in analytical sciences has been more significant and prominent in recent years than the past. This has really broadened our vistas and helped to develop new methods of analysis. In pharmacy analytical chemistry is responsible for developing sensitive, reliable and more accurate methods for the estimation of drug in pharmaceutical dosage form.

DRUG PROFILE

- **Name:** Dolutegravir
- Structure:

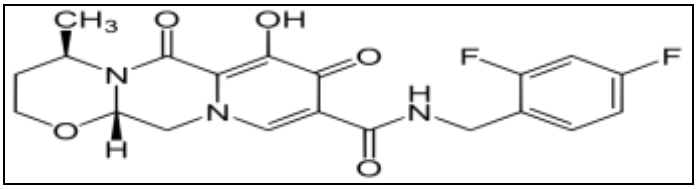

Figure 1 - Structure of dolutegravir

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Table1 - General Profile of Dolutegravir

Category	**Anti-retroviral Agent**
Chemical Name	(3S,7R)-N-[(2,4-difluorophenyl) methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo [8.4.0.0^ {3,8}] tetradeca-10,13-diene-13-carboxamide
Molecular Formula	$C_{20}H_{19}F_2N_3O_5$
Molecular Weight	419.38 g/mol
Description	White power
Solubility	Soluble in methanol
pKa	8.2
Melting point	190-193ºC

➢ **Pharmacological action:**

Dolutegravir is approved for use in a broad population of HIV-infected patients. It can be used to treat HIV-infected adults who have never taken HIV therapy (treatment-naïve) and HIV-infected adults who have previously taken HIV therapy (treatment-experienced), including those who have been treated with other integrase strand transfer inhibitors. Tivicay is also approved for children ages 12 years and older weighing at least 40 kilograms (kg) who are treatment-naïve or treatment-experienced but have not previously taken other integrase strand transfer inhibitors.

➢ **Pharmacodynamics:**

HIV-1 infected subjects on dolutegravir monotherapy demonstrated rapid and dose-dependent reduction of antiviral activity with declines of HIV-1 RNA copies per ml. The antiviral response was maintained for 3 to 4 days after the last dose.[3] The sustained response obtained in clinical trials indicates that dolutegravir has a tight binding and longer dissociative half-life providing it a high barrier to resistance.[A31343] The combination therapy (ripivirine and dolutegravir) presented the same viral suppression found in previous three-drug therapies without integrase strand transfer inhibitor mutations or ripivirine resistance.

PLAN OF WORK

- Estimation of Dolutegravir Drug in tablet dosage from will be done by following methods.

1. Selection of Drugs and Formulation

- By literature and market survey

2. Selection of analytical techniques

- Estimation by UV-Visible spectroscopy.
- Identification by IR Spectroscopy
- HPLC method

3. QbD approach Method development by RP-HPLC.

- Selection of preliminary HPLC conditions.
- ✔ Selection of mobile phase
- ✔ Selection of column
- ✔ Selection of Flow rate
- ✔ Selection of Column Temperature
- ✔ Selection of run time

4. Validation of proposed method.

- ✔ System suitability test
- ✔ Linearity and Range
- ✔ Accuracy
- ✔ Precision

a. Method precision
b. Intermediate precision (Ruggedness)

- ✔ Robustness
- ✔ Specificity

▪ **DRUG USED IN EXPERIMENT**

Table 2: Drug and drug product samples suppliers and manufacturers

Name of drug and drug product	Supplier and manufacturer by
Dolutegravir	Hetero drugs
Dolutegravir tablet 50mg	Hetero life science

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- **REAGENTS**

Table 3: List of Reagent

Sr.No	Chemical	Make
1	Water	Rankem
2	Acetonitrile	Merck life science
3	Methanol	Merck life science
4	Phosphoric acid 88%	Merck life science
5	Potassium dihydrogen phosphate	Merck life science
6	0.45 µ Nylon membrane disc filter	Mdi
7	0.45µ PVDF Syringe Filter	Mdi

- **METHOD**

1. UV SPECTROSCOPIC METHOD

➢ **Selection of solvent**

Diluent: Prepare mixture of water: methanol in the ratio of 50:50 v/v respectively, mix well and degas. Used as solvent for dissolving API

➢ **SELECTION OF WAVELENGTH**

Preparation of Drug Standard solution: An accurately weighed quantity about 10 mg of Dolutegravir standard was transferred to 100 ml volumetric flask. Add 70 ml of diluent, sonicate to dissolve and dilute up to the mark with diluent and mixed (100 ppm).

➢ **DETERMINATION OF λ MAX (SELECTION OF WAVELENGTH)**

The standard solutions were scanned separately between 400nm to 200nm. From the spectrum show high absorbance.

2. IDENTIFICATION BY IR SPECTROSCOPY

Dolutegravir 20 mg API was mixed individually properly then carefully triturated in a mortar pestle. At last, this mixture was kept in on a plate and IR spectrum was taken using the Diffused Attachment reflectance mode.

3.REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND OPTIMIZATION

The standard solution of Dolutegravir was used for method development trials to optimize the method for determination of Dolutegravir.

I. PREPARATION OF SOLUTION

➢ **Preparation of Buffer solution:**

Weigh and transfer about 1.36 gm of potassium dihydrogen phosphatewas added to 1000 mL HPLC water mix well. The pH was adjusted to 3.2 ± 0.05 with dilute orthophosphoric acid. Filter through 0.45µ nylon.

➢ **Preparation of Mobile phase:**

A mixture of Buffer Solution pH 3.2 and methanol in the ratio 55:45 v/v, was prepared, mixed and sonicated to degas used as mobile phase.

➢ **Preparation of diluent:**

Buffer pH 3.0: methanol (50:50%v/v) was used as diluent.

➢ **Preparation of Standard solution:**

Accurately weighed 50 mg of Dolutegravir as working/reference standard was transferred into 100 mL volumetric flask. About 30 mL of diluent was added and sonicated to dissolve. The solution was cooled to room temperature and made up to mark with diluent.

Further dilute 5 mL of stock solution of Dolutegravir was pipette out and transferred to 50 mL volumetric flask and made volume up to mark with Diluent(Concentration of Dolutegravir: 40 ppm).

➢ **Preparation of Sample preparation:**

Weigh and Transferred 5 intact tablet of Dolutegravirinto 250 mL volumetric flask. Added about 200 mL of diluent, sonicated for 25 minutes with intermittent shaking cool and dilute up to the mark with diluent and mix, allowed to settle for 15 min. centrifuge this solution at 5000 RPM. Further dilute 4 mL of this supernatant solution to 100 mL with diluents. Filtered through 0.45µ Nylon membrane syringe filter (Concentration of Dolutegravir: About 40 ppm).

II. CHROMATOGRAPHIC CONDITION

The standard solution of Dolutegravir API was used for method development. Different trials were planed and taken to optimize the method for determination of Dolutegravir.

➢ **Selection of Stationary phase:**

On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C18 bonded phase i.e. Inertsil

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ODS -3v C18 (250 mm X 4.6 mm), 5µm was selected.

➢ **Selection of Mobile Phase:**

The selection of mobile phase was done after assessing the solubility of drug in different solvent as well on the basis of literature survey and finally mobile phase was selected for is the mixture of Buffer Solution pH 3.0 and methanol in the ratio 60:40 v/v.

➢ **Selection of Detector and Detection wavelength:**

UV-visible 2487 detector was selected, as it is reliable and easy to set at the correct wavelength and 261 nm wavelengths was selected as detection wavelength.

➢ **Optimization of Chromatographic Parameters:**

Optimization in HPLC **was** the process of finding a set of conditions that adequately separate and enable the quantification of the analyte from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

➢ **Selection of oven temperature:**

An inclusion of column temperature (25°C) minimized day to day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

Table 4: Finalized Chromatographic Conditions

Column	Inertsil ODS -3V C18 (250 mm X 4.6 mm), 5µm
Mobile phase	Buffer pH 3.2: methanol (60:40)
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	261 nm
Column Temp.	30°C
Auto sampler Temp.	25°C
Run time	25.0 min.
Needle wash	Water: methanol (20:80 v/v)
Seal wash	Water: methanol (80:20 v/v)

II. RESULTS AND DISCUSSION

A simple, precise and economic RP-HPLC method was developed and validated for estimation of Dolutegravir in bulk and tablet dosage form. The method was validated as per ICH guidelines by using various validation parameters such as System suitability, Linearity, accuracy, precision and robustness.

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND OPTIMIZATION

1. Selection of Wavelength

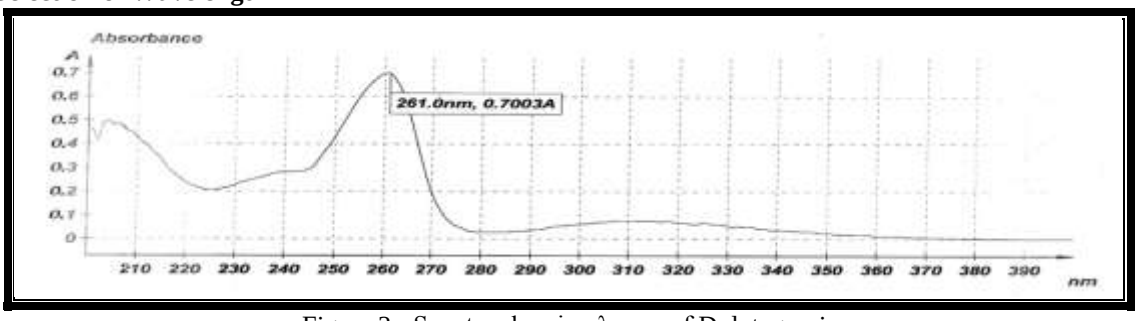

Figure 2 - Spectra showing λ max of Dolutegravir

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Table 5: Determination of λ max of Dolutegravir

Sr. No.	Wavelength (nm)	Absorbance
1.	261	0.7003 A

2. Identification by IR Spectroscopy:

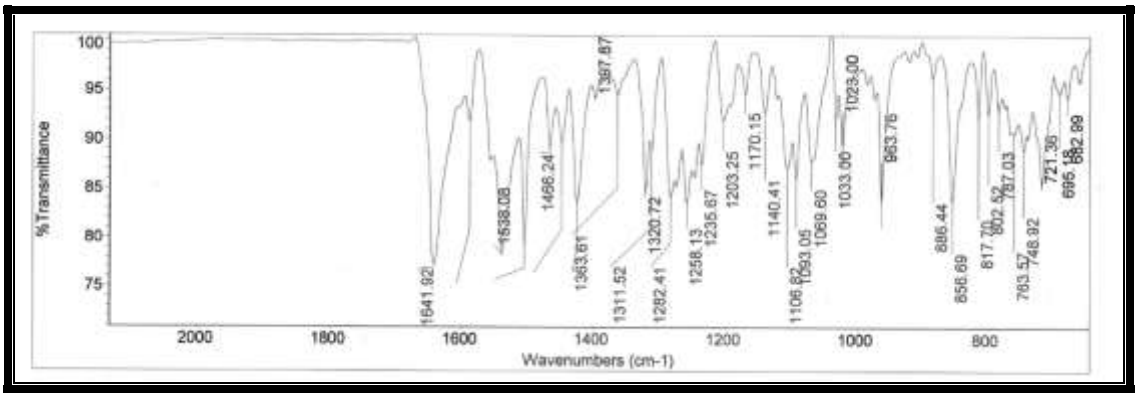

Figure 3 - FTIR spectrum of Dolutegravir Standard Solution

Table 6 - IR peak Assignment value of Dolutegravir

Standard IR Ranges (cm^{-1})	IR Ranges (cm^{-1})	Functional Group
1910-1690	1641.92	**C=O** Stretching
1385-1360	1363.61	**C-H** Bending
1390-1310	1311.52	**O-H** Bending
1342-1266	1282.41	**C-N** Stretching
1400-1000	1140.41	**C-F** Stretching
980-960	963.76	**C=C** Bending

3. Reverse Phase High Performance Liquid Chromatography Method Development
Different trials taken were as follows:
TRIAL: 1
Chromatographic Conditions:

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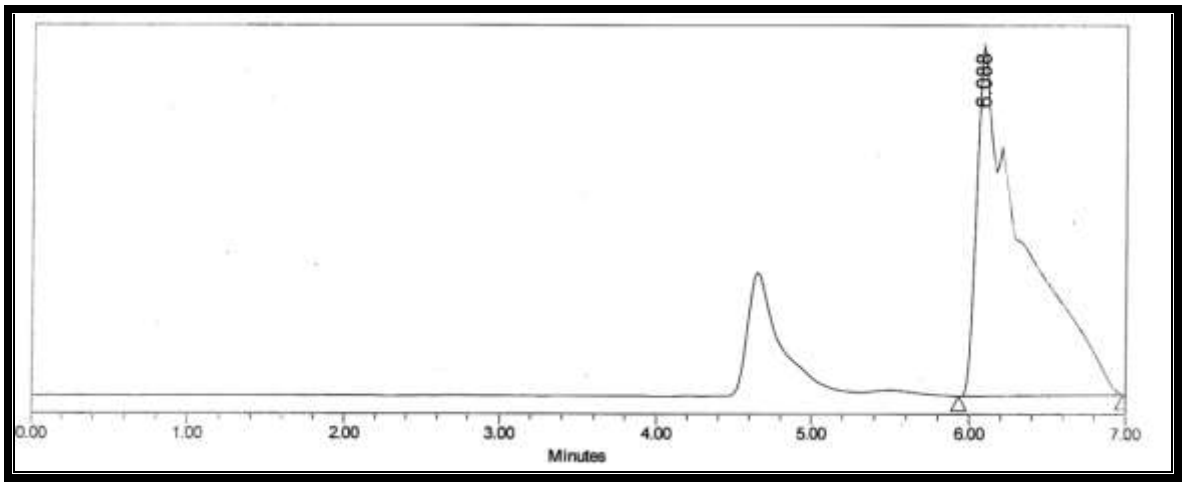

Figure 4 - Typical chromatogram for Trial- 1

Conclusion: Dolutegravir peak observed at 6.088 min, but method needs to be optimized.

TRIAL: 2

Chromatographic Condition:

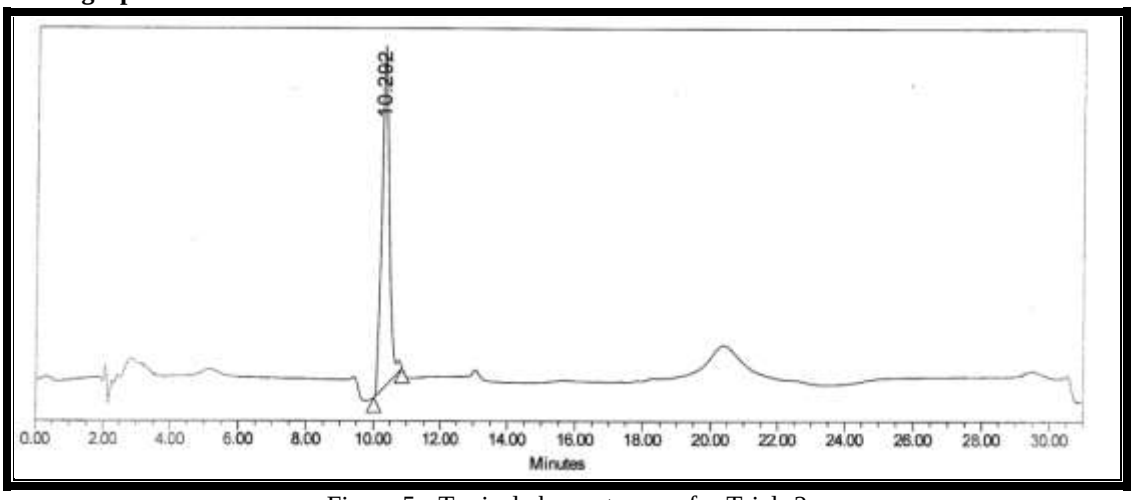

Figure 5 - Typical chromatogram for Trial- 2

Conclusion: -Dolutegravir peak observed at 10.292 min, but method needs to be optimized. Mobile phase needed to be changed further to increase the retention time.

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TRIAL: 3

Chromatographic Condition:

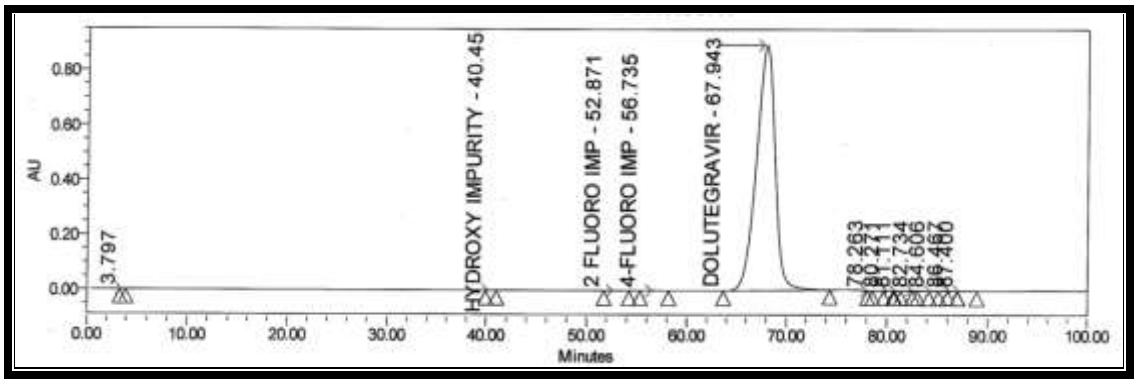

Figure 6 - Typical chromatogram for Trial- 3

Conclusion: -Dolutegravir peak observed at 67.943 min, but method needs to be optimized. Mobile phase needed to be changed further to decrease the retention time

TRIAL: 4
Chromatographic Condition:

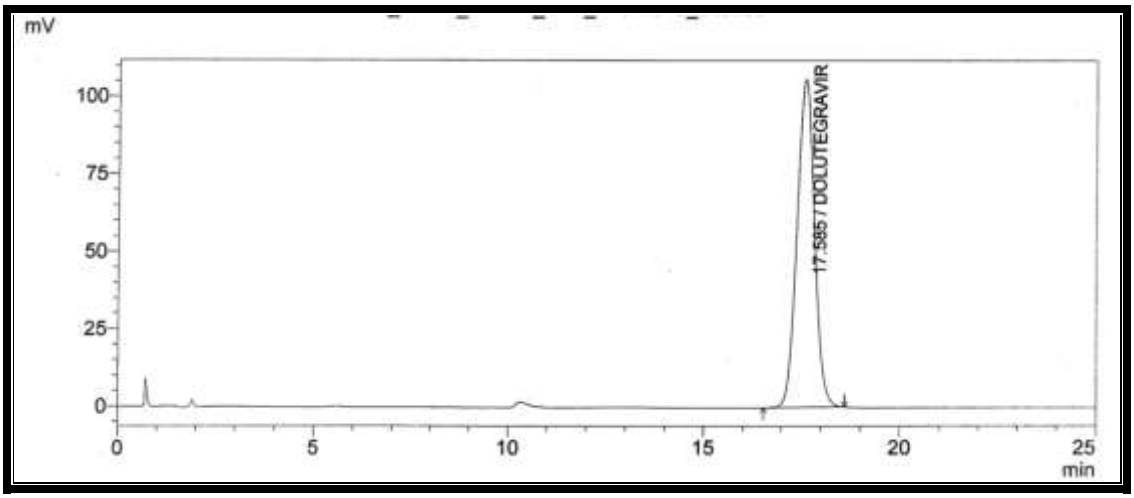

Figure 7 - Typical chromatogram for Trial- 4

Conclusion: -Keeping rest of the chromatographic conditions constant, Dolutegravirpeak was eluted at RT 17.585 min. peak shows peak purity. Theoretical plate, USP plate count, symmetry was found to be satisfactory. So, conditions of trial 4 were selected as optimized chromatographic conditions.

- **METHOD VALIDATION**

The following parameters were considered for the analytical method validation of title ingredients.

- System Suitability
- Specificity
- Linearity
- Accuracy
- Precision
  - System Precision
  - Method Precision
  - Intermediate Precision
- Robustness

1. SYSTEM SUITABILITY

System suitability test is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the

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chromatographic system are adequate for analysis to be done.

Table 7:System Suitability Test

Tailing factor	**1.2**
Theoretical plates	**8510**
S. No.	**Area**
1	1504857
2	1503223
3	1503927
4	1504994
5	1504461
6	1505139
Mean	1504434
% RSD	0.05

2. SPECIFICITY: (Identification, Interference & Peak Purity)
Inject Blank (Diluent), standard solution, impurity Solution, placebo solution and sample solution. The data obtained is summarized in Table 8.

Table 8: Specificity (Identification and Interference)

Sr. No.	**Component**	***RT (min)**	**Tailing factor**	**Purity angle**	**Purity threshold**
1	Blank	-	-	-	-
2	Placebo solution	-	-	-	-
3	Standard solution	16.462	1.2	0.573	0.858
4	Sample solution	16.511	1.1	0.507	0.854
6	DolutegravirRelated compound A	3.946	1.20	5.868	17.159
7	DolutegravirRelated compound B	4.823	0.96	11.288	24.567
8	DolutegravirRelated compound C	8.539	1.10	14.786	29.246
10	DolutegravirRelated compound E	2.170	1.01	2.718	7.679
12	DolutegravirRelated compound G	11.857	1.01	12.685	29.544

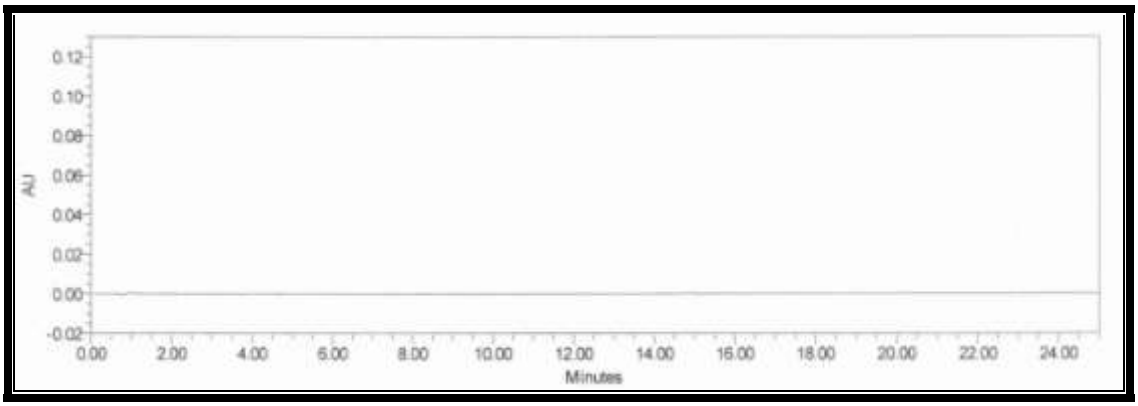

Figure 8- Chromatogram of Blank

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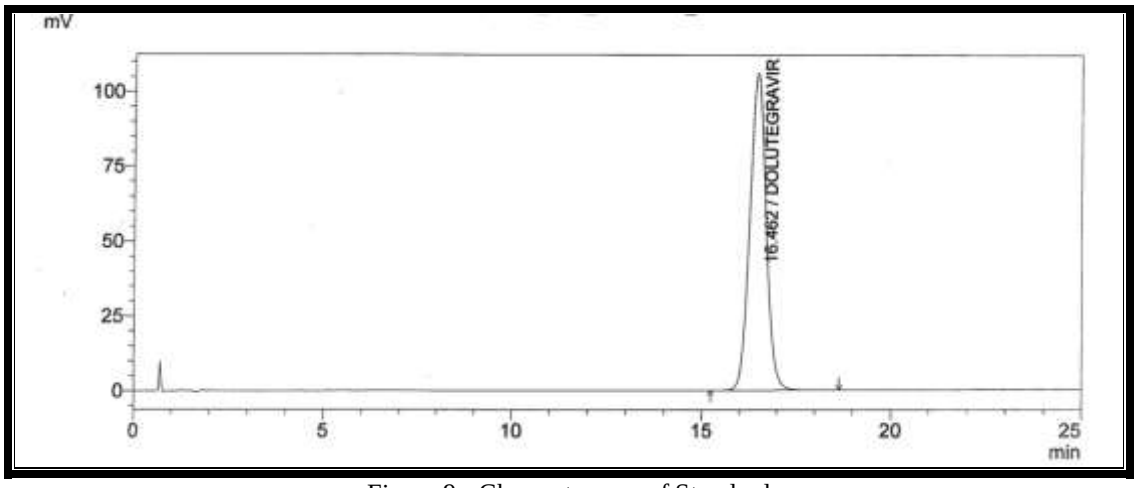

Figure 9 - Chromatogram of Standard

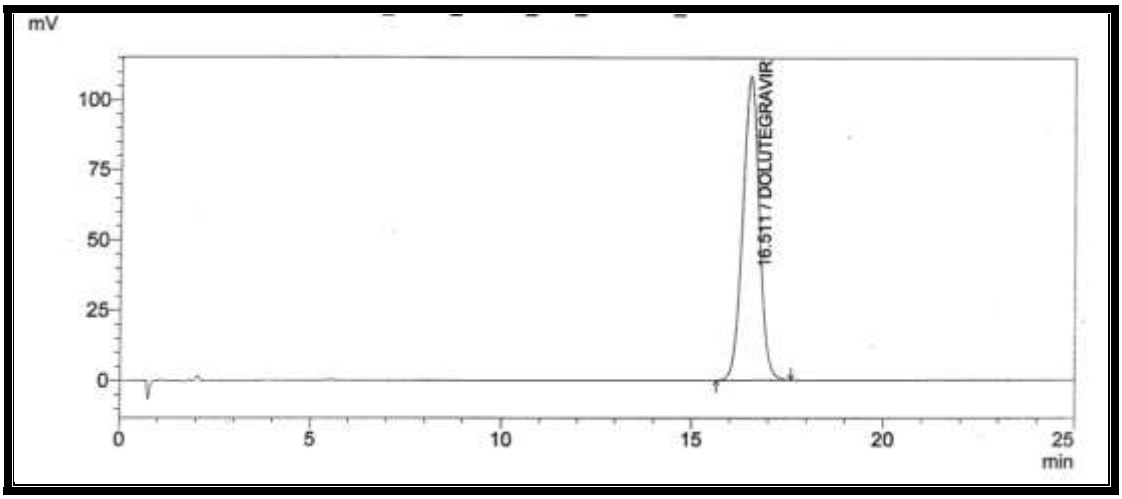

Figure 10 - Chromatogram of Sample

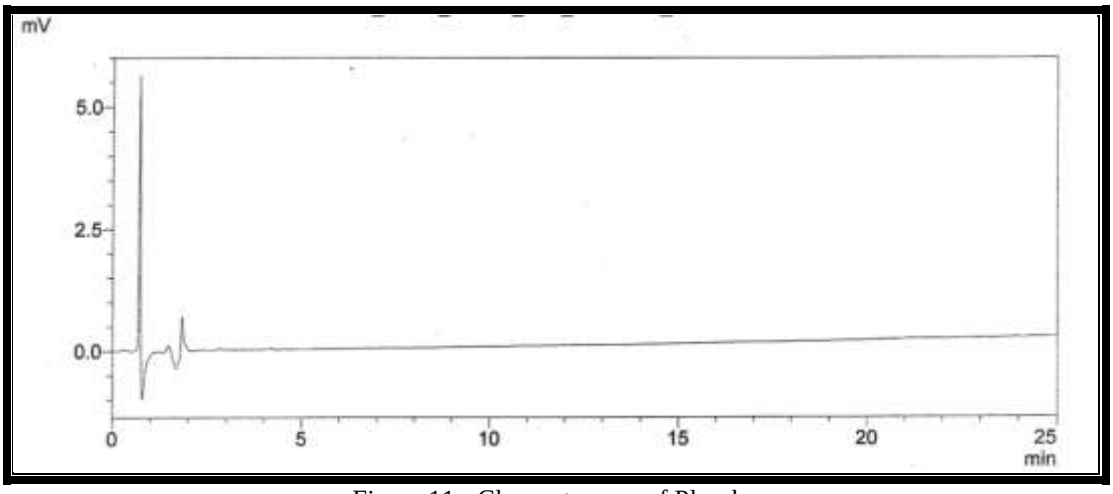

Figure 11 - Chromatogram of Placebo

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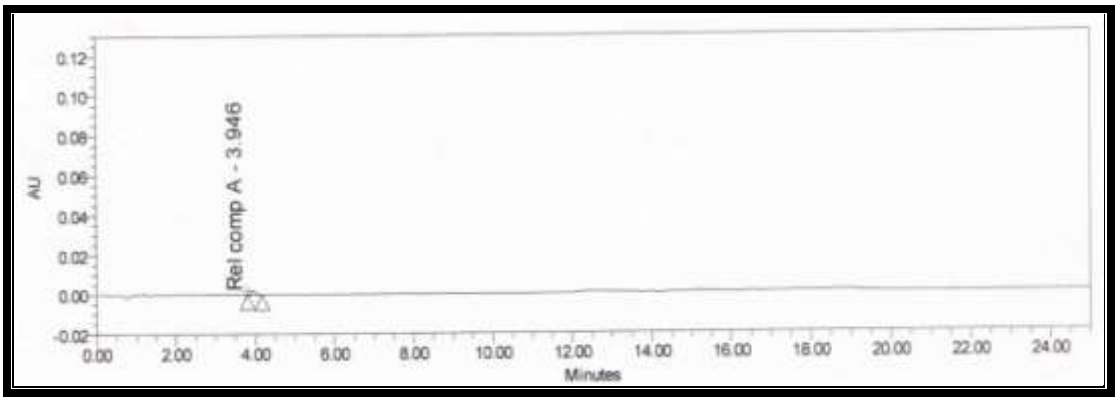

Figure 12 - Chromatogram of Dolutegravir Related compound A Impurity

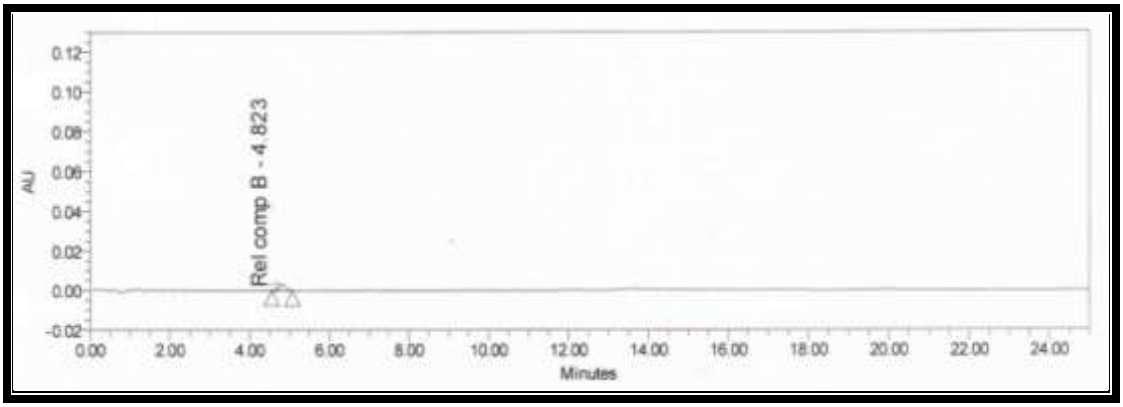

Figure 13 - Chromatogram of Dolutegravir Related compound B Impurity

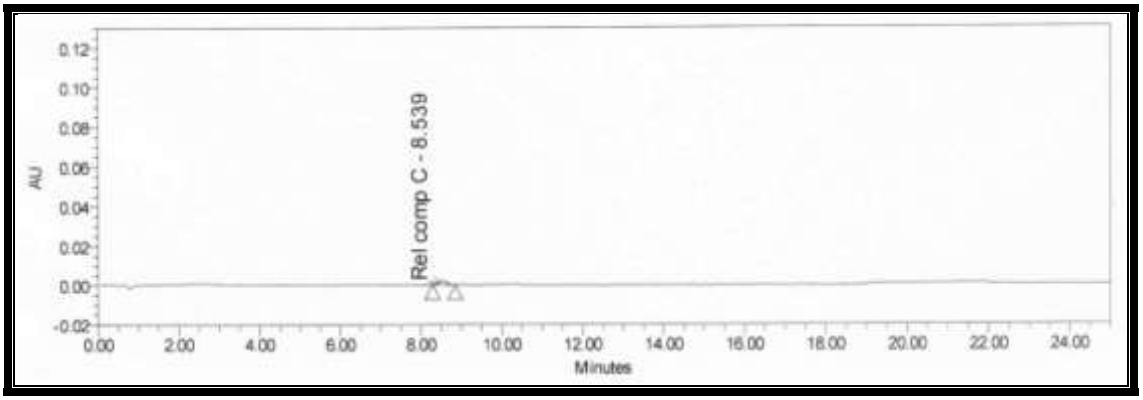

Figure 14 - Chromatogram of Dolutegravir Related compound C Impurity

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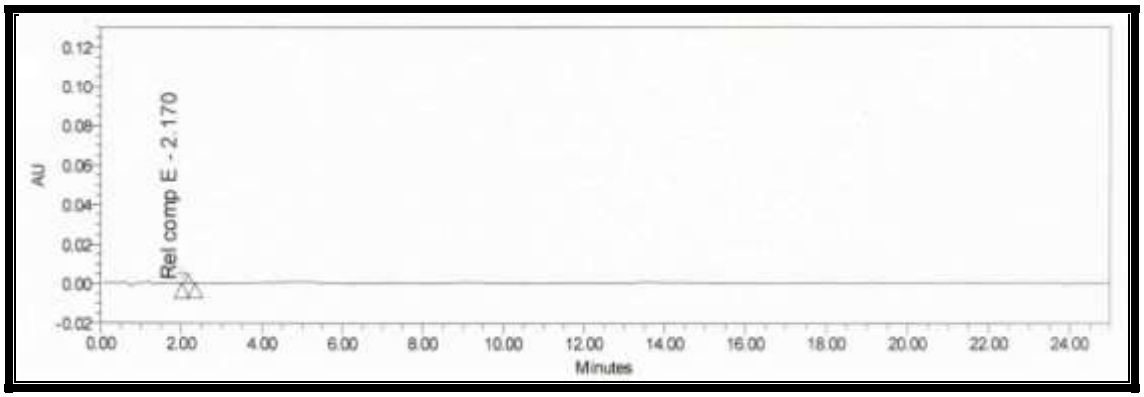

Figure 15 - Chromatogram of Dolutegravir Related compound E Impurity

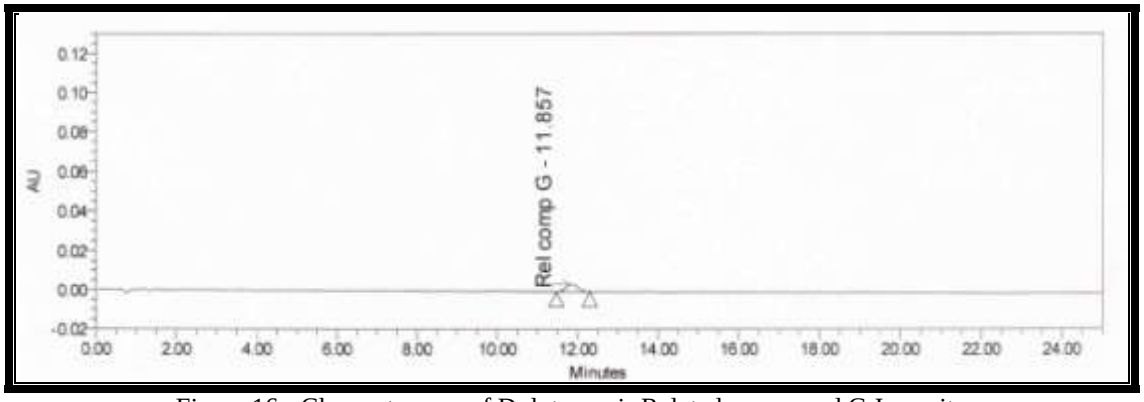

Figure 16 - Chromatogram of Dolutegravir Related compound G Impurity

3. LINEARITY

Linearity was evaluated in the range of 50% to 150% of the working concentration level. As the working concentration level of Dolutegravir.

Table 9: Linearity

Level (%)	**Concentration (ppm)**	**Response**		
		1	**2**	**Mean**
50	20	727622	728107	727865
75	30	1095876	1097581	1096729
100	40	1460057	1443791	1451924
125	50	1798255	1797370	1797813
150	60	2159058	2179246	2169152
Co-relation coefficient (r^2)				**0.9999**
SLOPE				**35837.46**
Y-INTERCEPT				**15233.1291**
WORKING LEVEL AREA				**1451921**
%LIMIT OF Y-INTERCEPT (± 5 OF WORKING LEVEL)				**1.050**

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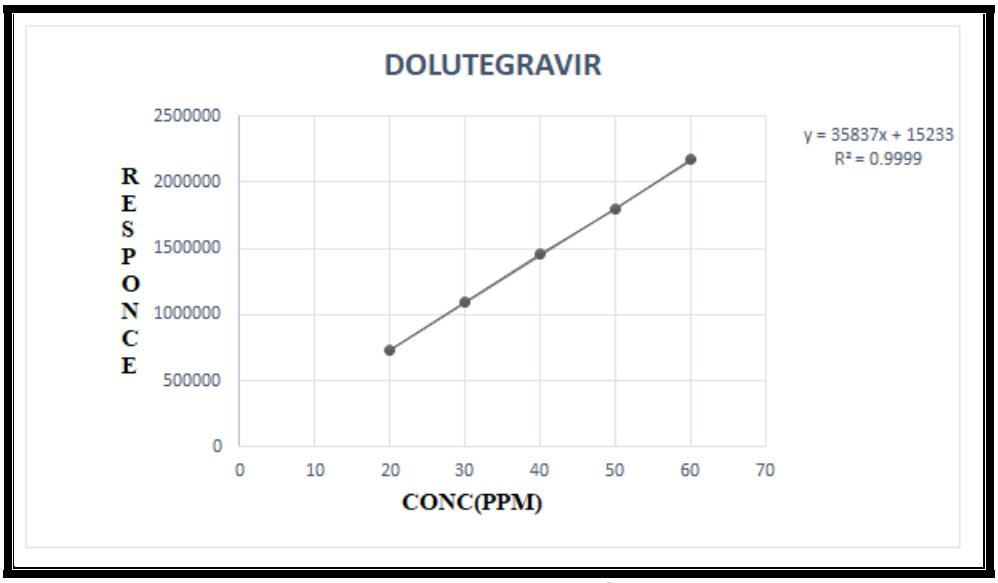

Figure 17 - Linearity plot

4.ACCURACY (Recovery):

Accuracy was evaluated three levels 50%, 100% and 150% of the working concentration level for Dolutegravir. As the working concentration level of Dolutegravir, each level prepared in triplicates.

Table 10: % Recovery

Level (%)	Theoretical concentration (mcg/mL)	% Recovery	Mean recovery%
50	20.78	100.8	100.3
	20.66	100.4	
	20.20	99.9	
100	40.78	101.4	101.5
	40.69	101.4	
	40.50	101.3	
150	60.99	101.5	101.3
	60.85	101.2	
	60.79	101.1	
Mean recovery			**101.0**

5. PRECISION

➢ **System Precision**

Single injection of blank (Diluent), Standard solution (six replicates) was injected on the system.

Tailing factor	**1.3**
Theoretical plates	**8436**
S. No.	**Area**
1	1512365
2	1502227
3	1500387
4	1504994

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5	1513861
6	1512389
Mean	1507704
% RSD	0.4

Table 11: System Precision

➢ **Method Precision:**

Single injection of blank (Diluent), Standard solution (six replicates) and sample solution (six preparations) was injected on the system.

Table 12: Method precision

Sample No.	**% Assay**
1	99.9
2	98.5
3	99.8
4	98.6
5	99.3
6	98.2
Mean	**99.05**
% RSD	**0.7**

➢ **Intermediate Precision:**

Five independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 13: Intermediate Precision

Parameter	**Method Precision(Analyst-I)**	**Intermediate Precision(Analyst-II)**
HPLC Instrument No.	**HPLC-001**	**HPLC-007**
Date of analysis	**XXX**	**XXX**
HPLC column No.	**LC-035**	**LC-020**
Sample No.	**% Assay**	
1	99.9	100.3
2	98.5	98.9
3	99.8	99.4
4	98.6	98.8
5	99.3	99.2
6	98.2	100
Mean	**99.05**	**99.43**
Average	**99.24**	
% RSD of all determinations	**0.7**	

6.ROBUSTNESS

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

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Table 14: Robustness

Parameters	Values	%Assay	Absolute difference
Control	As per method	100.4	-
Flow rate (± 0.1 mL/min)	0.9mL/min	100.4	0.0
	1.1mL/min	100.2	0.2
Change in Wavelength(± 5 nm)	256nm	100.4	0.0
	266 nm	98.8	1.7
Buffer pH(±0.2 unit)	pH 3.0	98.6	1.9
	pH 3.4	98.6	1.9
Column temperature (± 5°C)	35C	99.6	0.9
	25°C	99.8	0.7

SUMMARY

The results of analysis in this method were validated in terms of accuracy, precision, ruggedness, linearity. The method was found to be sensitive, reliable, reproducible, rapid and economic also.

Table 15: Summary of System suitability

Sr. No.	Parameters	Acceptance criteria	Result obtained		
1.0	System suitability The relative standard deviation of six replicate injections	NMT 2.0%	0.05		
	Tailing factor	NMT 1.5	1.2		
	Theoretical plates	NLT 2000	8510		
2.0 2.1	Specificity Identification	Results should be comparable with respect to the retention time.	RT of Standard: 16.462		
			RT of Sample	16.511	
2.2	Interference	Blank (Diluent), Placebo and known Impurities should not show any peak at the retention time of Dolutegravirpeak	Complies		
2.3	Peak purity	Standard and Sample peak should be pure at working concentration level. Purity angle should be less than purity threshold.	**Dolutegravir**	**Purity an**	**Purity Threshold**

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				gle	
			Standard	0.673	0.828
			Sample for	0.547	0.804

Sr. No	**Parameters**	**Acceptance criteria**	**Result obtained**	
4.0	Linearity and Range Correlation coefficient	NLT 0.990	0.9999	
	Y- intercept	Intercept y <± 2.0% of standard response	1.050	
5.0	Accuracy (Recovery)	Mean and Individual recovery for 50% to 150% should be in the range of 95.0%- 105.0%.		
			Level %	% Mean Recovery
			50	100.3
			100	101.5
			150	101.3
6.0 6.1	Precision System Precision	System suitability criteria should be fulfilled.	Complies	
6.2	Method precision	The RSD for % assay of six independent samples preparations: NMT 2.0%.		
			% Mean Assay	99.05
			% RSD	0.7

Sr. No	**Parameters**	**Acceptance criteria**	**Result obtained**	
6.3	Intermediate Precision (Ruggedness)	The RSD for % Assay of six independent samples preparation should not be more than 2.0%.		
		The cumulative % RSD for % assay of twelve independent	% Mean Assay	99.24

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		samples preparation of two analysts should not more than 2.0%.	% RSD	0.7

Sr. No	Parameters	Acceptance criteria	Result obtained
8.0	Robustness Change in Flow rate (± 0.1 mL/min) Change in Column temperature (± 5°C) Change in Wavelength (±2 nm) Buffer pH(±0.2 unit)	System suitability criteria should be fulfilled. The cumulative % RSD for % assay obtained in each modified condition should not be more than 2.0 when compared to the method precision.	Complies

III. CONCLUSION

RP-High Performance Liquid Chromatography (HPLC) Method

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Dolutegravir tablet formulation. HPLC Water2469 with Inertsil ODS -3v C18(250 mm X 4.6 mm), 5µm column and UV/PDA detector with empower pro Software was used for the study. The standard and sample solution of Dolutegravir were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

The mobile phase that was found to be most suitable was Buffer and Methanol, the wavelength 261 nm were selected for the evaluation of the chromatogram of Dolutegravir respectively. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture in water: methanol. This system gave good resolution and optimum retention time with appropriate tailing factor (<2).

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in tablet formulation.

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