INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Paper

Development and Validation of an HPLC Method for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and Pharmaceutical Dosage Forms

Baswaraju Aruna*, Macharla Rakesh, Jarupla Abhishek, Ulli Vaman Shiva, Durgam Shashank

Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, Telangana.

ARTICLE INFO

Published: 05 June 2026
Keywords:
Dolutegravir; Rilpivirine; RP-HPLC; Method development; Validation; ICH guidelines; Simultaneous estimation; Quality control
DOI:
10.5281/zenodo.20556264

ABSTRACT

A simple, precise, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and optimized for the simultaneous estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a YMC ODS C18 column with a mobile phase of phosphate buffer and methanol (35:65 v/v) at pH 3.5, yielding well-resolved peaks with satisfactory retention times and system suitability parameters. The method was validated according to ICH guidelines, demonstrating excellent linearity (10–50 µg/ml, correlation coefficients >0.999), precision (%RSD within acceptable limits), and accuracy (recovery close to 100%). Sensitivity was confirmed through low LOD and LOQ values. Robustness studies further established the reliability of the method. Overall, the developed RP-HPLC method is accurate, precise, linear, robust, and sensitive, making it suitable for routine quality control analysis of Dolutegravir and Rilpivirine in combined dosage forms. Its economical and time-efficient nature enhances applicability in pharmaceutical industries and research laboratories

INTRODUCTION

Pharmaceutical analysis is an essential discipline within pharmaceutical sciences that ensures the identity, purity, safety, and efficacy of drug substances and pharmaceutical formulations. It encompasses a wide range of analytical techniques used throughout drug development, manufacturing, and quality control processes. The primary objective of pharmaceutical analysis is to guarantee that drugs meet predefined specifications and regulatory requirements. **(1,2,3,5,6)**

***Corresponding Author:** Baswaraju Aruna
Address: *Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, Telangana..*
Email ✉: udayasri14@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Importance of High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques for the separation, identification, and quantification of components in complex mixtures. It is especially valuable in pharmaceutical analysis due to its ability to handle thermally unstable, non-volatile, and high molecular weight compounds. **(4,11,13,14)**

HPLC offers several advantages, including: **(4,13,14)**

- High resolution and selectivity
- Rapid analysis with reproducible results
- Capability to analyze multiple components simultaneously
- High sensitivity for trace-level detection
- Compatibility with a wide range of detectors

INTRODUCTION TO HPLC

HPLC is an advanced form of column chromatography that operates under high pressure to increase the efficiency and speed of the separation process. It has become a standard method for analyzing complex mixtures in various industries, particularly pharmaceuticals. **(4,13,14)**

Principles of HPLC: The principle of HPLC is based on the partitioning of components between a stationary phase (usually packed inside a column) and a mobile phase (solvent that moves through the column). The different affinities of each component for the stationary and mobile phases cause them to elute at different times, known as retention times. **(4,14)**

Types of HPLC:

1. **Normal Phase HPLC:** Utilizes a polar stationary phase and a less polar mobile phase.
2. **Reverse Phase HPLC (RP-HPLC):** The most common type, uses a non-polar stationary phase and a polar mobile phase.
3. **Ion Exchange HPLC:** Separates ions and polar molecules based on their charge.
4. **Size Exclusion HPLC:** Also known as gel permeation or gel filtration, separates molecules based on size.
5. **Affinity HPLC:** Uses the specific interactions between one kind of solute molecule and a second molecule that is immobilized on a stationary phase. **(4,11,13,14)**

Applications of HPLC:

1. **Pharmaceuticals:** Used for the analysis of active pharmaceutical ingredients (APIs), excipients, and formulations.
2. **Environmental Monitoring:** Detects pollutants and contaminants in water, air, and soil.
3. **Food Industry:** Analyzes food components for quality control and to ensure compliance with regulations.
4. **Clinical Research:** Assists in the study of biological samples, such as plasma, for drug development and disease markers.
5. **Industrial Applications:** Used in the production of chemicals and other materials to monitor process streams and ensure product purity.

HPLC is valued for its accuracy, precision, and ability to analyze a wide range of compounds. Its versatility makes it an indispensable tool in scientific research and quality control laboratories worldwide. **(4,5,11,13)**

System Suitability:

According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples

constitute an integral system that can be evaluated as a whole. The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application. **(9)**

Similar to the analytical method development, the system suitability test strategy should be revised as the analysts develop more experience with the assay. **(3,9)**

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (%RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method. These parameters are measured during the analysis of system suitability "sample" that is a mixture of main components and expected by-products. Below Table lists the terms to be measured and their recommended limits obtained from the analysis of the system suitability sample as per current FDA guidelines on "Validation of Chromatographic Methods". **(3,9)**

Table 4. System Suitability Parameters and Recommendations

Parameter	Recommendation
Capacity Factor (k')	The peak should be well-resolved from other peaks and the void volume, generally k'>2.0
Repeatability	RSD $\leq$ 1% for N $\geq$ 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T of $\leq$ 2
Theoretical Plates (N)	N > 2000

Accuracy: Defined as the closeness of agreement between the actual (true) value and mean analytical value obtained by applying the test method a number of times. Accuracy is acceptable if the difference between the true value and mean measured value does not exceed the RSD values obtained for repeatability of the method. **(1,12)**

Precision: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). This involves **(1,12)**

a) Repeatability
b) Reproducibility
c) Intermediate precision

$$\% RSD = 100\ S/X$$

Where, S = Standard deviation

X = Mean

It is determined at three levels.

Linearity: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope of regression line. It is determined by series of three to six injections of five of more standards. **(1,12)**

Limit of Detection (LOD): It is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. **(1,12)**

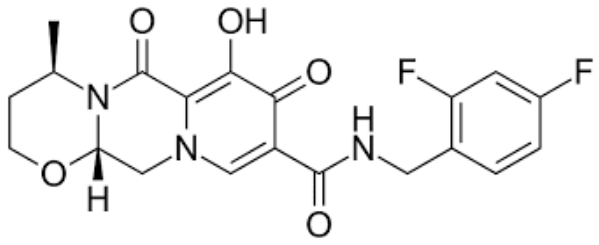
In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level.

S/N = 2/1 or 3/1

LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level.
S/N = 10/1

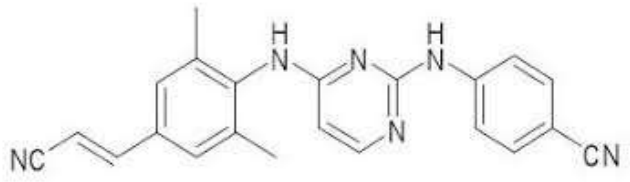
Robustness: It is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in procedure. It provides an indication about variability of the method during normal laboratory conditions. **(1,12)**

MOLECULAR STRUCTURE OF DOLUTEGRAVIR



Molecular Formula	C20H19F2N3O5 **(17,18)**
Molecular Weight	419.45 g/mol **(17)**
IUPAC Name	(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 **(17,18)**
ChemSpider ID	25067956 **(18)**
Density	1.45 g/cm³ **(18)**
Boiling Point	998.8 °C (at 760 mmHg) **(18)**
Vapour Pressure	2.15 × 10^-9 Pa (at 25 °C) **(18)**
Flash Point	605.8 °C **(18)**
Refractive Index	1.63 **(18)**
Polar Surface Area	193.3 Å² **(17)**
LogP (Octanol/Water)	3.49 **(17)**
Generic Name	Dolutegravir **(16)**
Brand Names	Tivicay, Juluca (combination with rilpivirine) **(20)**
Drug Category	Antiretroviral, Integrase strand transfer inhibitor (INSTI) **(16,19)**
Indications	HIV-1 infection (treatment-naive and treatment-experienced adults and children) **(19,20)**
Pharmacology	Inhibits HIV-1 integrase, preventing viral DNA integration into host genome **(16,19)**
Potency	Highly potent, with IC50 values ranging from 0.05 to 0.5 nm **(16)**
Tolerability	Generally well-tolerated, with common adverse effects including insomnia, headache, and diarrhea **(16,20)**
Contraindications	Hypersensitivity to dolutegravir or any component of the formulation **(20)**
Adverse Effects	Common: insomnia, headache, diarrhea; Less common: hypersensitivity reactions, liver enzyme elevations **(16,20)**
Availability	Available in oral tablet and film-coated tablet formulations, as well as a powder for oral suspension **(20)**

MOLECULAR STRUCTURE OF RILPIVIRINE



. HCl

Molecular Formula	C22H18N6 **(22,23)**
Molecular Weight	366.42 g/mol **(22)**
IUPAC Name	4-{[4-({4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile **(22,23)**
ChemSpider ID	44457598 **(23)**
Density	1.23 g/cm3 **(23)**
Boiling Point	567.8 °C (1053.8 °F) **(23)**
Vapour Pressure	0.0 mmHg (20 °C) **(23)**
Flash Point	282.3 °C (540.1 °F) **(23)**
Refractive Index	1.65 **(23)**
Polar Surface Area	102.39 Å2 **(22)**
LogP (Octanol/Water)	4.45 **(22)**
Generic Name	Rilpivirine **(21)**
Brand Names	Edurant, Juluca (combination with dolutegravir) **(24)**
Drug Category	Antiretroviral, Non-nucleoside reverse transcriptase inhibitor (NNRTI) **(21,25)**
Indications	HIV-1 infection (treatment-naive and treatment-experienced adults and children) **(24,25)**
Pharmacology	Inhibits HIV-1 reverse transcriptase, preventing viral replication **(21,25)**
Potency	Highly potent, with IC50 values ranging from 0.27 to 0.34 Nm **(21)**
Tolerability	Generally well-tolerated, with common adverse effects including depression, insomnia, and rash **(21,24)**
Contraindications	Hypersensitivity to rilpivirine or any component of the formulation Co-administration with proton pump inhibitors, rifampicin, rifapentine, carbamazepine, oxcarbazepine, phenobarbital, phenytoin, St. John's Wort **(24,25)**
Adverse Effects	Common: depression, insomnia, rash Less common: severe skin and hypersensitivity reactions, liver enzyme elevations **(21,24)**
Availability	Available in oral tablet formulation, 25 mg **(24)**
Mechanism of Action	1. Binds to HIV-1 reverse transcriptase **(21)** 2. Inhibits reverse transcription, preventing viral replication **(21,25)** 3. Prevents integration of viral DNA into host genome **(21,25)**

MATERIALS AND METHODS:

List of Proposed Materials:

S. No.	Chemicals/standards and reagents	Grade	Make	Used for the estimation of drugs
1	Phosphate buffer	HPLC	Qualigens	1. Dolutegravir and Rilpivirine
2	Acetic acid	HPLC	Qualigens	1. Dolutegravir and Rilpivirine
3	Water	HPLC	Qualigens	For all drugs
4	Acetonitrile	HPLC	Qualigens	For all drugs
5	Methanol	HPLC	Rankem	For all drugs

Equipment's and instruments used in the study:

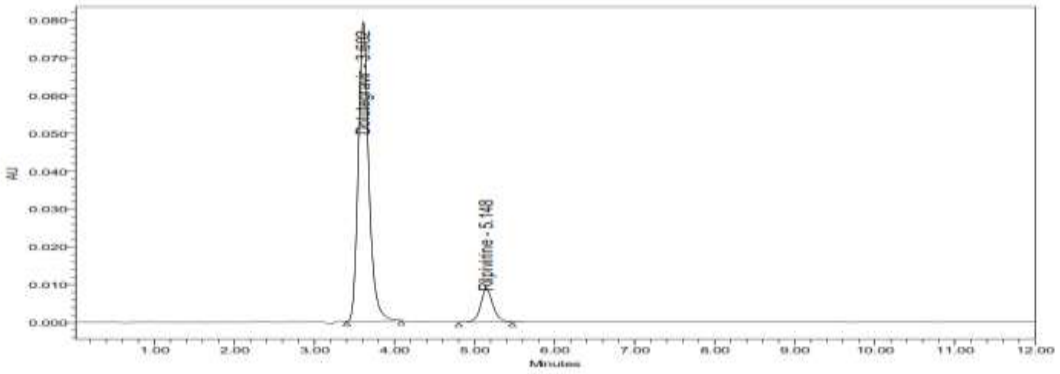
S.No.	Equipment	Model/Type	Manufacturer
1	Electronic Balance	SAB2032	SCALETEC
2	Ultra-Sonicator	SE60US	LABMAN SCIENTIFIC INDIA
3	Thermal Oven	i-THERM A17782	DWARAKA SCIENTIFIC
4	pH Meter	ORION STAR A111	THERMOSCIENTIFIC
5	Filter Paper	0.45 microns	MILLIPORE
6	HPLC System	WATERS 2690 SEPARATION MODULE	WATERS

Optimization of Column: YMC ODS (4.6*250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Equipment : High performance liquid chromatography equipped with Auto Sampler and PDA detector

Column : YMC ODS (4.6*250mm, 5µm)
Buffer : Phosphate buffer
P_H : 3.5
Mobile phase : 35% buffer: 65% Methanol
Flow rate : 1.0 ml per min
Wavelength : 250 nm
Injection volume : 20 µl
Run time : 12 min.



S.No	Name	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	Dolutegravir	3.602	214546	8021578	4.57	1.06	4596
2	Rilpivirine	5.148	34475	124577		1.2	3153

System Suitability: Tailing factor for the peaks due to Dolutegravir and Rilpivirine in Standard solution should not be more than 2.0. Theoretical plates for the Dolutegravir and Rilpivirine peaks in Standard solution should not be less than 2000

Acceptance criteria of System Suitability:

1) Tailing factor should be less than 2
2) Theoretical Plates should be above 2000

Calculation: (For Dolutegravir and Rilpivirine)

$$\%\,Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\,weight}{Label\,Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim mg/ml.

RESULTS AND DISCUSSION

TRIAL - 1

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA

Temperature : Ambient

Column : Platisil C_{18}, (250×4.6mm, 5µm)

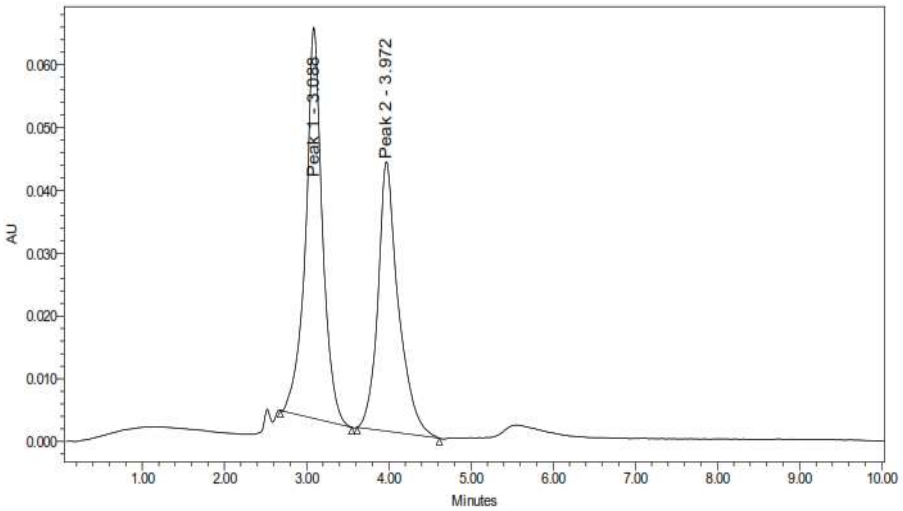
Mobile phase : 65% Methanol: 35% NaH2PO4 PH-4

Flow rate : 0.9 ml per min

Wavelength : 250 nm

Injection volume : 10 µl

Run time : 10 min.



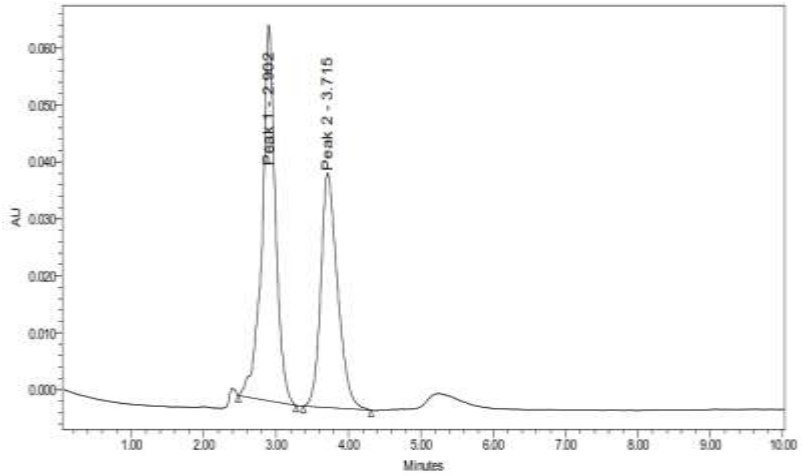
TRAIL 2

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA

Temperature : Ambient

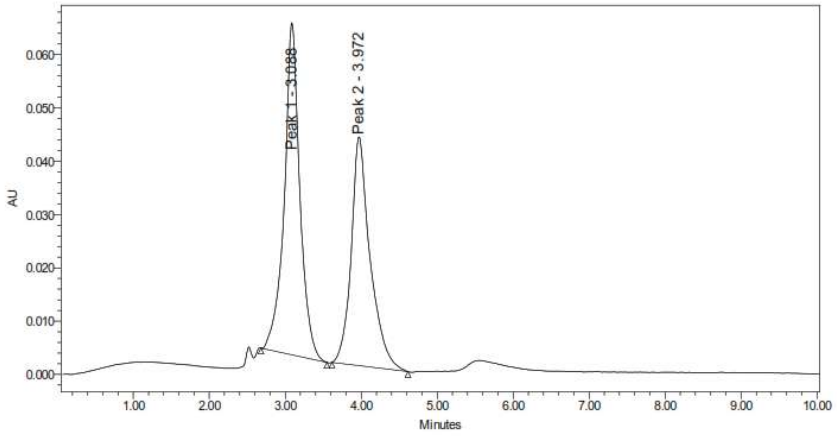
Column : Spurcil C18, (250×4.6mm, 5µm)

Mobile phase NaH2PO4 PH-4.5	:	65% Methanol: 35%	Wavelength	:	250 nm
			Injection volume	:	10 µl
Flow rate	:	0.8 ml per min	Run time	:	10 min.



TRAIL 3

Instrument used : High performance liquid chromatography equipped with Auto Sampler and PDA			Mobile phase NaH2PO4 PH-4	:	65% Methanol: 35%
			Flow rate	:	0.9 ml per min
			Wavelength	:	250 nm
Temperature	:	Ambient	Injection volume	:	10 µl
Column (250×4.6mm, 5µm)	:	Inertsil,	Run time	:	10 min.



1 VALIDATION PARAMETERS:

1.1 ASSAY:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

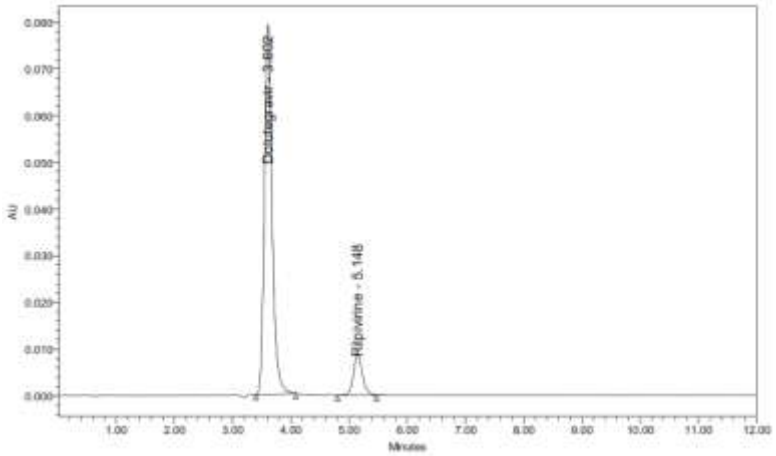


Figure 1: Chromatogram for Standard

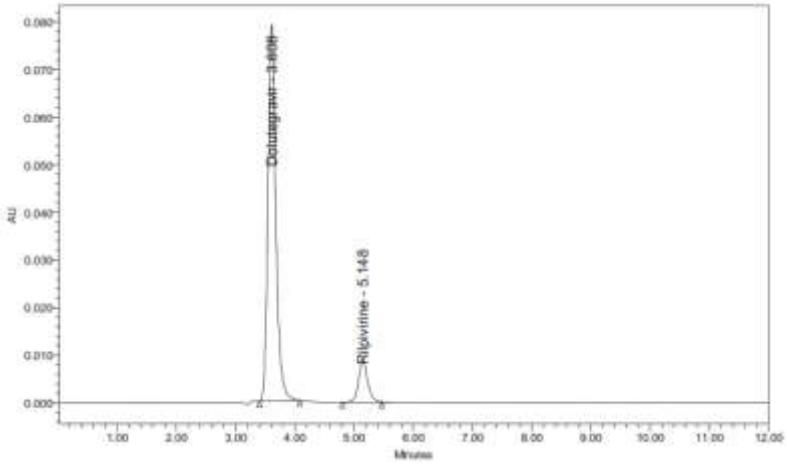


Figure 2: Chromatogram for Sample

Table 1: Results of Assay for Dolutegravir and Rilpivirine

S.No	Name (STD)	RT(min)	Area (µV sec)	Height (µV)	Resolution	USP tailing	USP plate count
1	Dolutegravir	3.608	12867	8021578	4.7	1.06	3246
2	Rilpivirine	5.148	34475	124577		1.08	68369
S.No	Name (Sample)	RT(min)	Area (µ V sec)	Height (µV)	5.01	USP tailing	USP plate count
1	Dolutegravir	3.608	214258	8021578		1.25	3465
2	Rilpivirine	5.148	33983	124577		1.06	6942

	Label Claim (mg)	% Assay
Dolutegravir	**50 mg**	**99.6%**
Rilpivirine	**25mg**	**98.0%**

2 LINEARITY:

The linearity range was found to lie from 10µg/ml to 50µg/ml of Dolutegravir and Rilpivirine and chromatograms are shown below.

Table 2: Area of different concentration of Dolutegravir and Rilpivirine

S. No	Dolutegravir	
	Concentration (µg/ml)	Area
1	20	71596
2	40	143965
3	60	213269
4	80	275985
5	100	347416

S. No	Rilpivirine	
	Concentration (µg/ml)	Area
1	10	10786
2	20	21469
3	30	32652
4	40	42586
5	50	53512

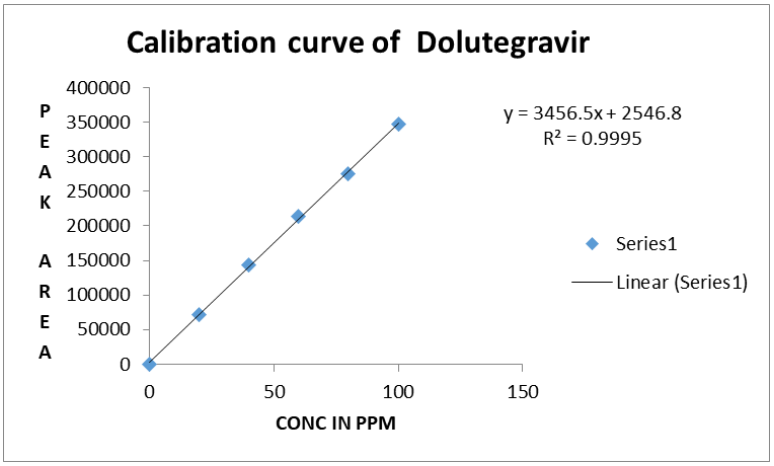


Figure 3: Calibration graph for Dolutegravir

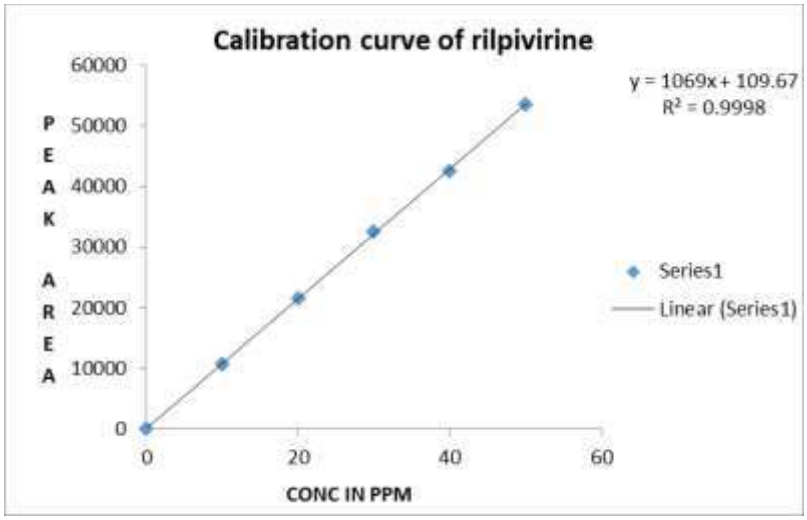


Figure 4: Calibration graph for Rilpivirine

Table 4: Analytical performance parameters of Dolutegravir and Rilpivirine

Parameters	Dolutegravir	Rilpivirine
Slope (m)	6913	534.49
Intercept (c)	2546.8	109.67
Correlation coefficient (R^2)	0.9995	0.9998

Results of Precision for Dolutegravir and Rilpivirine

Injection	Dolutegravir Area	Rilpivirine Area
Injection-1	214652	33321
Injection-2	214985	33658
Injection-3	214423	33756
Injection-4	214145	33249
Injection-5	214021	33943
Injection-6	214362	33145
Average	214213	33397
Standard Deviation	214400.1	33495.57
%RSD	329.4786	293.9069

Table 5: Results for variation in mobile phase composition for Dolutegravir and Rilpivirine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results of Dolutegravir	
		USP Plate Count	USP Tailing
1	**10% less(54ml)**	3075	1.05
2	***Actual(60ml)**	3069	1.08
3	**10% more(66ml)**	3053	1.02

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results of Rilpivirine	
		USP Plate Count	USP Tailing
1	**10% less(54ml)**	6947	1.04
2	***Actual(60ml)**	6991	1.07
3	**10% more(66ml)**	6939	1.2

CONCLUSION

The developed RP-HPLC method is accurate, precise, linear, robust, and sensitive for the simultaneous determination of Dolutegravir and Rilpivirine. All validation parameters including system suitability, precision, accuracy, linearity, LOD, LOQ, and robustness were found to be within acceptable limits, confirming the suitability of the method for routine quality control analysis. The method is economical, time-efficient, and reproducible, making it highly suitable for regular laboratory use in pharmaceutical industries and research laboratories for the analysis of these drugs in combined dosage forms.

REFERENCES

1. International Council for Harmonisation (ICH). ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.
2. International Council for Harmonisation (ICH). ICH Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva: ICH; 2003.
3. United States Food and Drug Administration (FDA). Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics. Silver Spring (MD): FDA; 2015.
4. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid

Chromatography. 3rd ed. Hoboken (NJ): John Wiley & Sons; 2010.
5. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Belmont (CA): Cengage Learning; 2007.
6. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. 7th ed. New Delhi: CBS Publishers; 2004.
7. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. 5th ed. Mumbai: Himalaya Publishing House; 2002.
8. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers; 2007.
9. United States Pharmacopeia (USP). USP 43–NF 38. Rockville (MD): United States Pharmacopeial Convention; 2020.
10. European Medicines Agency (EMA). Guideline on Bioanalytical Method Validation. London: EMA; 2011.
11. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Hoboken (NJ): John Wiley & Sons; 2007.
12. Swartz ME, Krull IS. Analytical Method Development and Validation. New York: Marcel Dekker; 2012.
13. Dong MW. Modern HPLC for Practicing Scientists. Hoboken (NJ): John Wiley & Sons; 2006.
14. Meyer VR. Practical High-Performance Liquid Chromatography. 5th ed. Chichester: John Wiley & Sons; 2010.
15. British Pharmacopoeia Commission. British Pharmacopoeia. London: The Stationery Office; 2020.
16. DrugBank Online. Dolutegravir: drug information, mechanism of action, pharmacology and pharmacokinetics. Available from: https://go.drugbank.com
17. PubChem Database. Dolutegravir; CID: 54726191. National Center for Biotechnology Information, U.S. National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov
18. ChemSpider. Dolutegravir: chemical structure and physicochemical properties; ChemSpider ID: 25067956. Royal Society of Chemistry. Available from: http://www.chemspider.com
19. World Health Organization (WHO). Dolutegravir (DTG): antiretroviral drug profile and clinical use in HIV treatment. Geneva: WHO; Available from: https://www.who.int

HOW TO CITE: Baswaraju Aruna, Macharla Rakesh, Jarupla Abhishek, Ulli Vaman Shiva, Durgam Shashank, Development and Validation of an HPLC Method for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and Pharmaceutical Dosage Forms, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 6, 1344-1355, https://doi.org/10.5281/zenodo.20556264