Development and Validation of a Reverse-Phase HPLC Method for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Combined Pharmaceutical Dosage Forms

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ABSTRACT

A simple, precise, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Dolutegravir and Rilpivirine in combined pharmaceutical dosage forms. Chromatographic separation was achieved using a Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of potassium phosphate buffer (pH 4.5) and methanol in a 50:50 v/v ratio, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 257 nm. The retention times for Dolutegravir and Rilpivirine were approximately 3.30 min and 5.74 min, respectively, with resolution > 12 and tailing factors < 1.4. The method was validated as per ICH Q2(R1) guidelines and demonstrated linearity in the ranges of 10–60 µg/mL ($r^2 > 0.99$) for Dolutegravir and 5–30 µg/mL ($r^2 > 0.999$) for Rilpivirine. Accuracy, expressed as recovery, was within 98.5–101.2% for both drugs, and %RSD values for precision were $< 2\%$. The method showed specificity with no interference from excipients and robustness under small deliberate changes in chromatographic conditions. The validated method is suitable for routine quality control analysis of fixed-dose antiretroviral formulations.

Keywords: RP-HPLC, Dolutegravir, Rilpivirine, Validation, Antiretrovirals, Quality assurance

INTRODUCTION

The global burden of HIV/AIDS continues to drive research toward effective and well-tolerated treatment options. Among the antiretroviral agents, Dolutegravir, an integrase strand transfer inhibitor, and Rilpivirine, a non-nucleoside reverse transcriptase inhibitor, have shown excellent efficacy, safety profiles, and improved patient compliance when used in combination therapy [1,2]. These drugs are often formulated together in a fixed-dose combination tablet to simplify treatment regimens and reduce pill burden [3].

Chemical Properties Of Dolutegravir

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]oxazine-9-carboxamide)

Molecular Formula: $C_{20}H_{19}F_2N_3O_5$

Molar mass: 419.38 g/mol

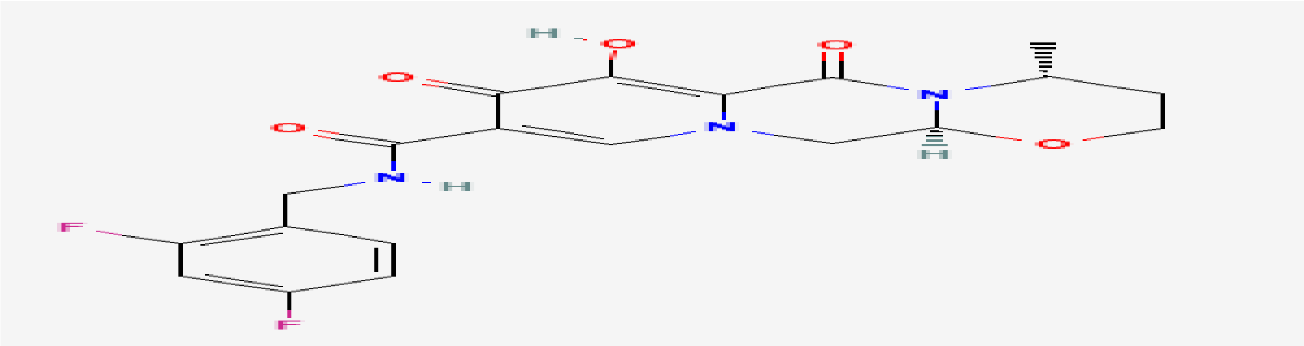

Fig.1: Structure Of Dolutegravir

Chemical Properties Of Rilpivirine

IUPAC Name: (4-[[4-[4-[(*E*)-2-cyanoethenyl]-2,6-dimethylanilino]pyrimidin-2-yl]amino]benzonitrile)

Molecular Formula : $C_{22}H_{18}N_6$

Molar Mass: 366.428 g/mol

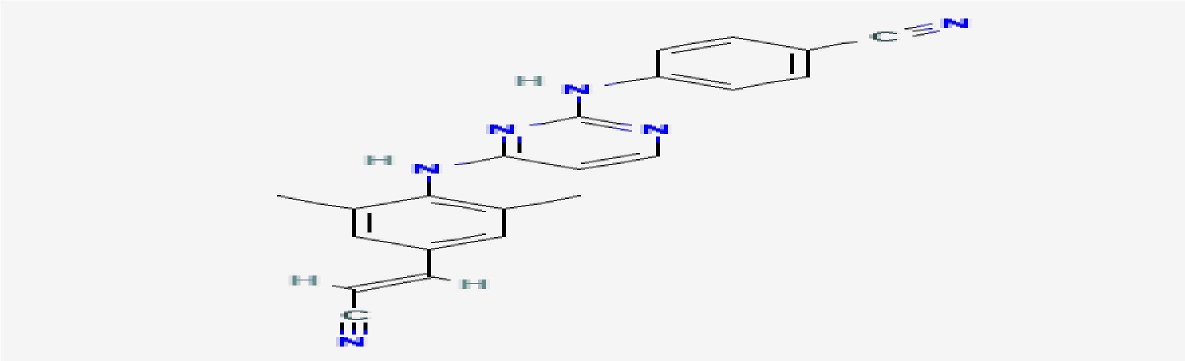

Fig.2: Structure Of Rilpivirine

The simultaneous quantification of multiple active pharmaceutical ingredients in combined dosage forms poses analytical challenges, requiring precise and validated methodologies. High-performance liquid chromatography (HPLC) is widely regarded as a preferred analytical tool due to its accuracy, sensitivity, and ability to handle complex mixtures [4,5]. Specifically, reverse-phase HPLC (RP-HPLC) is extensively used in pharmaceutical analysis owing to its flexibility, reproducibility, and compatibility with both hydrophilic and hydrophobic compounds [6].

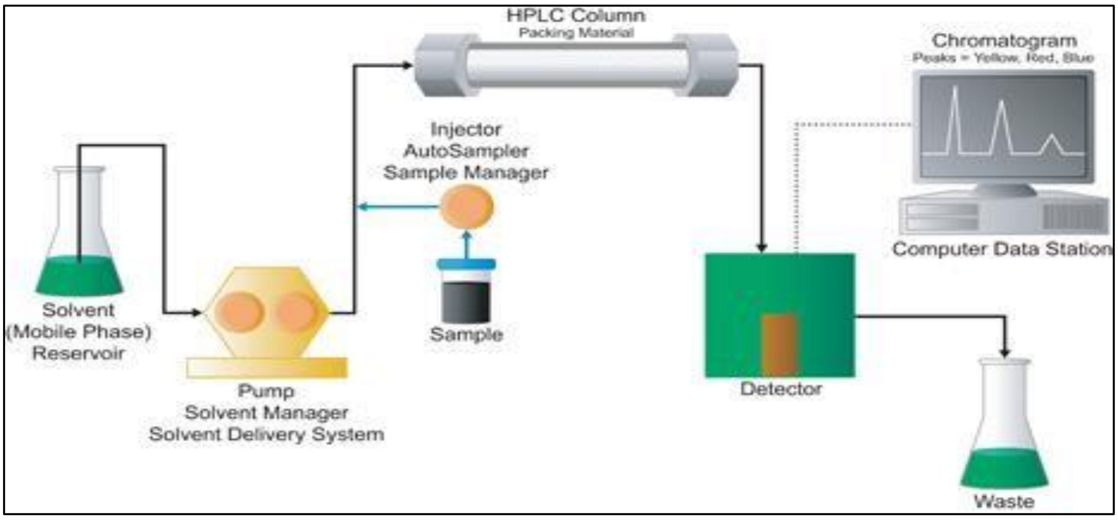

Fig. 3: Block diagram of HPLC

Although numerous analytical methods have been reported for individual estimation of Dolutegravir and Rilpivirine [7,8,9], there remains a lack of simple and robust RP-HPLC methods for their simultaneous determination in a single run, especially in combined pharmaceutical dosage forms. Addressing this gap, the current study aims to develop and validate an efficient RP-HPLC method that complies with ICH Q2(R1) guidelines [10], ensuring specificity, accuracy, precision, linearity, and robustness.

METHODOLOGY

Materials and Reagents

Dolutegravir was obtained from Emcure Pharmaceuticals Ltd., and **Rilpivirine** was sourced from Bharat Pharmaceuticals. The fixed-dose combination formulation (Juluca® tablets) containing 50 mg of Dolutegravir and 25 mg of Rilpivirine was used as the test sample. All reagents used were of analytical or HPLC grade, including methanol, acetonitrile, potassium dihydrogen phosphate, and water, which were procured from Merck Specialties Pvt. Ltd., Mumbai.

Instrumentation

Chromatographic analysis was performed using a **Shimadzu LC-20AT** HPLC system equipped with a binary pump, manual injector, UV detector (SPD-20A), and **Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µm)**. Data acquisition and analysis were performed using the associated Shimadzu software. Additional instruments included a digital pH meter and ultrasonic bath for solution degassing.

Selection of Detection Wavelength

To determine the optimal detection wavelength, standard solutions of Dolutegravir (50 µg/mL) and Rilpivirine (25 µg/mL) were scanned over the 200–400 nm range using a UV-Visible spectrophotometer. The overlay UV spectrum showed that both compounds exhibited significant absorbance at **257 nm**, which was selected as the detection wavelength .

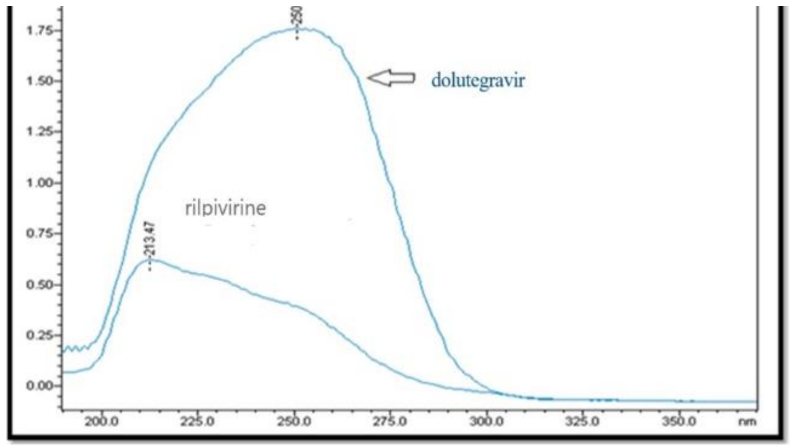

Fig.4 : UV Spectra of dolutegravir (50 ppm) and rilpivirine (25 ppm) in Methanol

Selection of Mobile Phase

Trial contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried. On the basis of various trails the mixture of Buffer (Potassium Phosphate) at pH 4.5: Methanol (50:50), at 1.0 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

Table 1: Trials are summarizing in following table.

Sr No	Mobile Phase	Remark
1	Water: Methanol (30:60)	One peak observed
2	Water: Methanol (40:60)	Peak of Dolutegravir Confirmed
3	Water: Methanol (60:40)	Retention time reduced
4	Water: Methanol (20:80)	Retention time reduced but second peak did not find
5	Methanol (100)	Still second peak did not find
6	Water: Acetonitrile (50:50)	No second peak observed
7	Water: Acetonitrile (30:70)	Still second peak did not observe
8	Water: Methanol: Acetic acid (30:70:0.1)	Second peak observed even by using an Acetic acid
9	Water: Acetonitrile: Acetic acid (30:70:0.1)	Second peak observed even by using an Acetic acid with Acetonitrile
10	Water (pH 4.0): Methanol (50:50)	Peak of Rilpivirine Confirmed
11	Water (pH 4.0): Methanol: TEA (50:50:0.1)	Peak shape of Rilpivirine did not become sharp by using TEA
12	Buffer (pH 4.0): Methanol (50:50)	Peak shape became good but peak of Dolutegravir Found at solvent peak time
13	Buffer (pH 4.5): Methanol (50:50)	Run time Increased but Both peaks follow SST Parameters
15	Buffer (pH 4.5): Methanol (30:70)	Run time Increased

Trial 15:

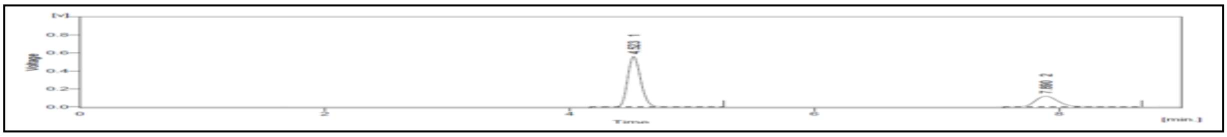

Fig.5: HPLC Chromatogram of Rilpivirine 25ppm and Dolutegravir 50ppm in Buffer (pH 4.5): Methanol (30:70)

Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, Buffers(potassium dihydrogen) in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. On the basis of various trials the mixture of Buffer(Potassium phosphate, pH 4.5): Methanol (50:50)

Table 2: Parameters of Dolutegravir and Rilpivirine

Parameters	**Dolutegravir**	**Rilpivirine**
Retention Time	3.303	5.740
Theoretical Plates	8048	8112
Asymmetry	1.238	1.353
Resolution	12.117	

Chromatographic Conditions

After a series of trials with various mobile phases and flow rates, the following optimized chromatographic conditions were finalized for simultaneous estimation:

- **Mobile Phase**: Potassium phosphate buffer (pH 4.5): Methanol (50:50, v/v)
- **Flow Rate**: 1.0 mL/min
- **Detection Wavelength**: 257 nm
- **Injection Volume**: 20 µL
- **Column Temperature**: Ambient
- **Run Time**: Approximately 10 minutes

These conditions provided sharp, symmetrical peaks for both drugs with adequate resolution and minimal tailing .

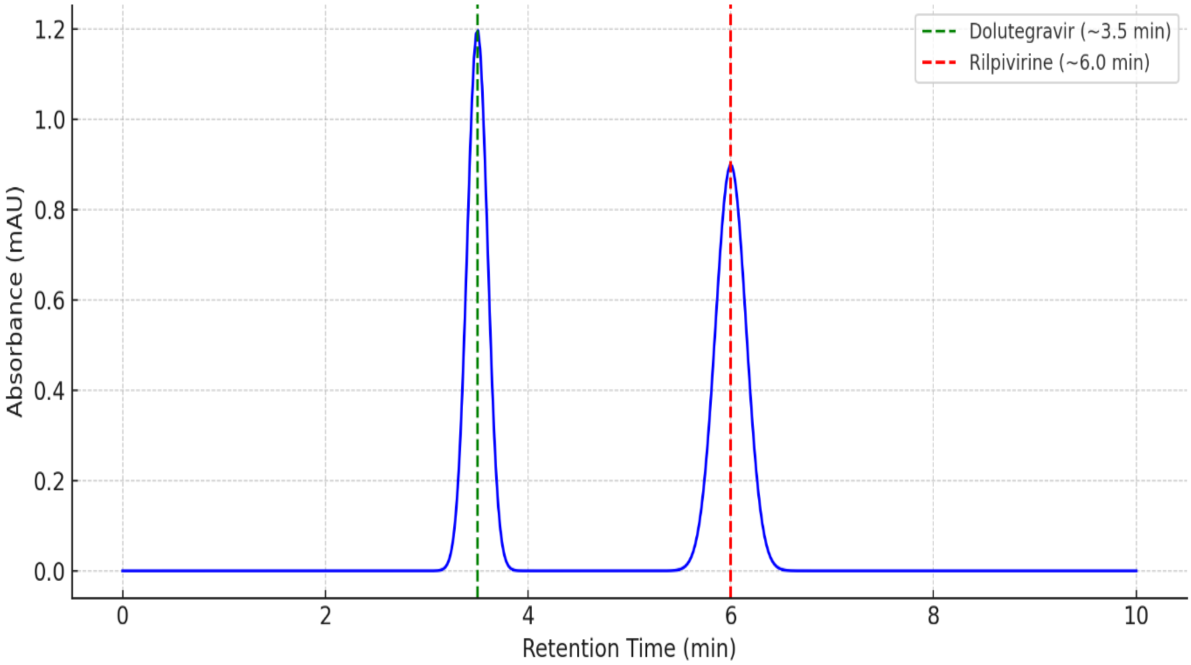

Fig.6:HPLC Chromatogram of Dolutegravir and Rilpivirine

Above is the HPLC chromatogram showing two distinct peaks:

- **Dolutegravir** at ~3.5 minutes
- **Rilpivirine** at ~6.0 minutes

These peaks indicate good resolution and selectivity using the optimized mobile phase and chromatographic conditions.

Preparation of Standard Solutions

- **Dolutegravir Standard**: Accurately weighed 50 mg of Dolutegravir was dissolved in methanol and diluted to 100 mL. A 1 mL aliquot was further diluted to 10 mL to achieve a final concentration of **50 µg/mL**.
- **Rilpivirine Standard**: 25 mg of Rilpivirine was dissolved similarly and diluted to achieve **25 µg/mL**.

Sample Preparation

One tablet of Juluca was weighed and crushed to a fine powder. An amount equivalent to one tablet was transferred into a volumetric flask, dissolved in methanol, and sonicated for 15 minutes. The solution was filtered using Whatman No. 42 filter paper and appropriately diluted to match the calibration range.

RESULTS AND DISCUSSION

Method Optimization

Several mobile phase combinations were tested using different ratios of methanol, acetonitrile, and buffer to achieve optimal separation of Dolutegravir and Rilpivirine. The finalized method using a 50:50 mixture of potassium phosphate buffer (pH 4.5) and methanol provided well-resolved peaks with retention times of approximately 3.5 minutes for Dolutegravir and 6.0 minutes for Rilpivirine (Fig. 2). Peak symmetry, resolution, and baseline separation were acceptable, meeting system suitability criteria.

Linearity

The calibration curves for both drugs were linear over the concentration ranges of 10–60 µg/mL for Dolutegravir and 5–30 µg/mL for Rilpivirine. The correlation coefficients (R^2) were greater than 0.999, indicating excellent linearity.

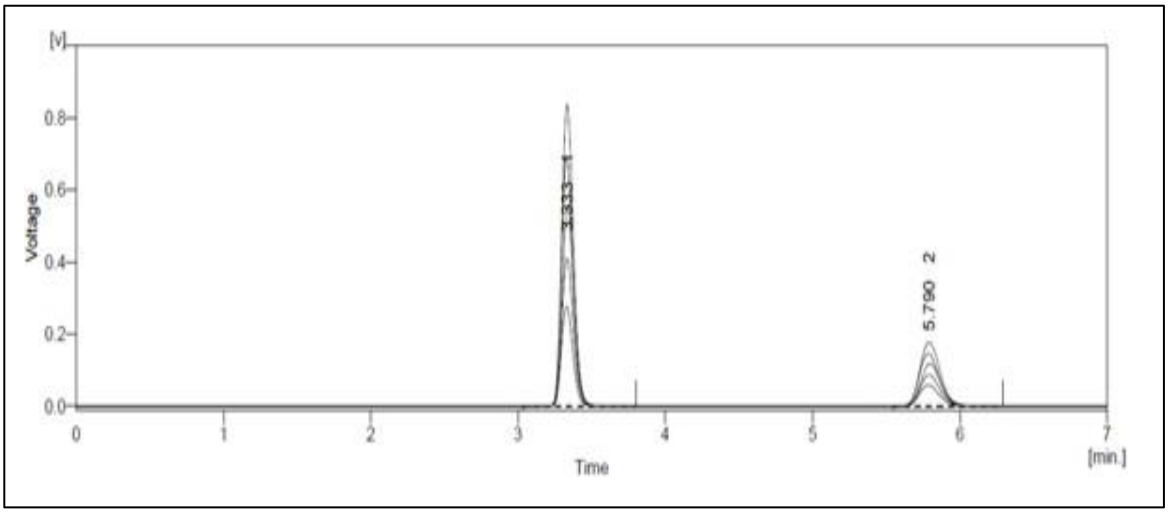

Fig.7: Overlay chromatogram of different concentrations of mixtures of Rilpivirine and Dolutegravir

Accuracy and Precision

Accuracy was confirmed by recovery studies at three concentration levels (80%, 100%, and 120%). The mean recoveries ranged between 98.5% and 101.2% for both drugs, meeting ICH guidelines. Intra-day and inter-day precision studies showed %RSD values below 2%, reflecting good method reproducibility.

Specificity

The method showed no interference from excipients present in the tablet formulation. Peaks were sharp, symmetrical, and free from tailing or overlap, confirming specificity.

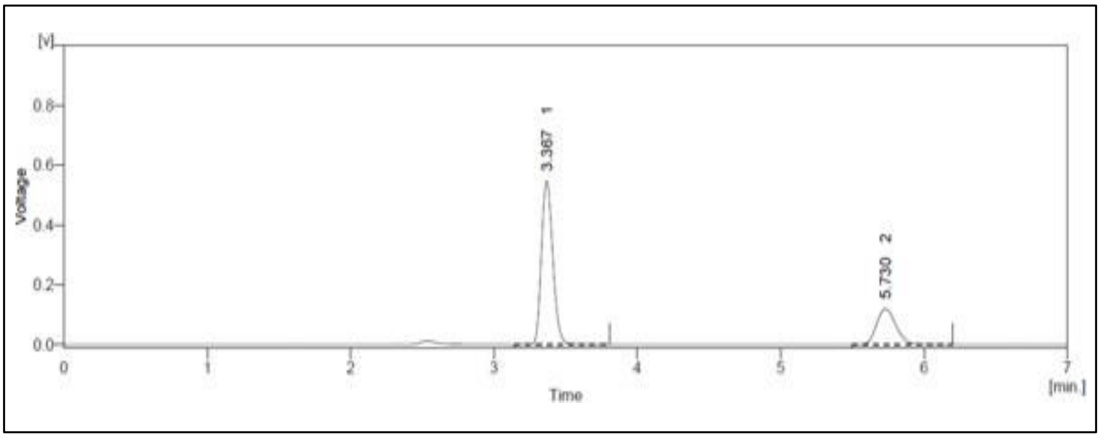

Fig.8:-Chromatogram of Dolutegravir and Rilpivirine sample

LOD and LOQ

- **Dolutegravir**: LOD = 0.2 µg/mL, LOQ = 0.6 µg/mL
- **Rilpivirine**: LOD = 0.05 µg/mL, LOQ = 0.15 µg/mL

These values demonstrate the method's sensitivity.

Robustness

Minor variations in pH, flow rate, and detection wavelength had no significant effect on retention times or peak areas, confirming robustness.

Summary Table Of Validation Parameters

Table 3: Summary of Validation Parameters

Parameter	**Dolutegravir**	**Rilpivirine**	**Typical acceptance criteria**	**Outcome**
System suitability	RT:3.30min Plates:8048 Tailing(As): 1.238 Resolution between peaks: >12	RT: 5.74 min RT: 5.740 min; Plates: 8112; Tailing (As): 1.353	Plates > 2000; Tailing < 2; Rs > 2	Pass
Linearity	10-60 µg/mL, r² > 0.99	5–30 µg/mL, r² > 0.999	r² ≥ 0.999;visual residual randomness	Pass
Range	10–60 µg/mL	5–30 µg/mL	Covers 80–120% of target with acceptable accuracy & precision	Adequate
Accuracy (Recovery)	98.5–101.2% (80, 100, 120%)	98.5–101.2% (80, 100, 120%)	98–102% (typical for assay of drug products)	Pass

Precision (Repeatability & Intermediate)	%RSD < 2%	%RSD < 2%	%RSD ≤ 2%	Pass
Specificity	No interference from excipients	No interference from excipients	Placebo/blank do not interfere with analyte peaks	Pass
LOD / LOQ	0.2 / 0.6 µg/mL	.05 / 0.15 µg/mL	Reported & justified	Meets
Robustness	No significant impact with small, deliberate changes in pH, flow, λ	No significant impact with small, deliberate changes in pH, flow, λ	No critical change in SST / results	Pass

CONCLUSION

The present study successfully established a validated RP-HPLC method for the simultaneous determination of Dolutegravir and Rilpivirine in combined dosage forms. The optimized chromatographic conditions—potassium phosphate buffer (pH 4.5) and methanol (50:50 v/v), 1.0 mL/min flow rate, and detection at 257 nm—provided well-resolved, symmetrical peaks with high theoretical plate counts and short run times. Validation results confirmed excellent linearity, accuracy, precision, specificity, sensitivity, and robustness, meeting all ICH Q2(R1) acceptance criteria. Owing to its simplicity, reproducibility, and cost-effectiveness, the proposed method is recommended for routine assay, stability studies, and quality control testing of fixed-dose antiretroviral combinations containing Dolutegravir and Rilpivirine.

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