

Stability Indicating Reverse Phase-High Performance Liquid Chromatography Method for Simultaneous Estimation of Cabotegravir and Rilpivirine

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ABSTRACT

Background: For the estimation of cabotegravir and rilpivirine in the bulk and pharmaceutical dosage form, a stability-indicating reverse-phase high-performance liquid chromatography method was developed and validated using an inertsil C₁₈ (150 x 4.6 mm, 5 µm) column. At a flow rate of 1.0 ml/min, a mobile phase containing a mixture of 0.01N ammonium acetate buffer (pH 3) and acetonitrile (65:35, v/v) was passed over the column. The column temperature was set at 30°C. A photodiode array detector was used at the wavelength of 257 nm. **Results:** Retention times of cabotegravir and rilpivirine were found to be 2.250 min and 2.823 min, respectively. The calibration curves were linear over the concentration range of 10-60 µg/ml and 15-90 µg/ml for cabotegravir and rilpivirine, respectively with a correlation coefficient (*R*²) of 0.999. The percent relative standard deviation (% RSD) for precision and robustness studies was found to be < 2%. The mean % recovery was obtained as 100.71 % and 100.01 % for cabotegravir and rilpivirine, respectively. The degradation during stability studies was more in the presence of oxidative conditions. **Conclusion:** The developed method was found to be simple, rapid, and economical and can be applied successfully for simultaneous estimation of cabotegravir and rilpivirine in regular analysis.

Keywords: Cabotegravir, Rilpivirine, RP-HPLC, Method Validation, Stability studies.

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Received: 25-08-2022;

Revised: 18-01-2023;

Accepted: 01-04-2023.

INTRODUCTION

Cabotegravir, chemically known as (3S,11aR)-N-((2,4-difluorophenyl)methyl)-6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11a-hexahydrooxazolo(3,2-a)pyrido(1,2-d)pyrazine-8-carboxamide (Figure 1a) is a second-generation integrase inhibitor, used for HIV treatment.¹ Rilpivirine, chemically designated as 4-{{4-({4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl}amino)pyrimidin-2-yl}amino}benzotrile (Figure 1b), belongs to the category of Anti-HIV drug.² Cabotegravir blocks the strand transfer of viral DNA and thereby inhibits the replication, whereas rilpivirine also hinders the viral replication but as a non-nucleoside reverse transcriptase inhibitor.³

Literature review reveals that till now there is only one Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

method reported for simultaneous estimation of cabotegravir and rilpivirine with stability studies.⁴ However, the reported method has lower sensitivity and is less economical. The other method reported, used Reverse Phase Ultra-Performance Liquid Chromatography (RP-UPLC) for simultaneous estimation of cabotegravir and rilpivirine, however, not performed the forced degradation studies.⁵ There are three HPLC methods reported for simultaneous estimation of rilpivirine and dolutegravir.⁶⁻⁸

The proposed HPLC method in comparison to the reported HPLC method is summarized in Table 1. It shows that the proposed method is more sensitive and economical than the reported method.⁴

MATERIALS AND METHODS

Chemicals

HPLC grade water and acetonitrile were purchased from Merck Pvt. Ltd., Mumbai. Ammonium acetate and acetic acid of analytical grade were also procured from Merck Pvt. Ltd., Mumbai. The working standards of cabotegravir and rilpivirine were provided as a gift sample from Spectrum Pharma Research Pvt. Ltd., Hyderabad.



DOI: 10.5530/ijper.57.3s.87

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Table 1: Comparison between the proposed method and the reported method.

Parameter	Proposed HPLC method		Reported HPLC method ⁴	
Mobile Phase	0.01 N Ammonium acetate buffer: acetonitrile (65:35 v/v)		0.1 % Formic acid: acetonitrile (20: 80 v/v).	
RT (minutes)	Cabotegravir	Rilpivirine	Cabotegravir	Rilpivirine
	2.250	2.823	3.942	2.05
Theoretical plate count	4926	962	3852	2079
% RSD	0.9	0.4	1.90	1.33
LOD ($\mu\text{g/ml}$)	0.13	0.16	0.25	0.38
LOQ ($\mu\text{g/ml}$)	0.38	0.48	0.83	1.24
Concentration range	10-60 $\mu\text{g/ml}$	15-90 $\mu\text{g/ml}$	20-300 g/ml	30-450 g/ml
Run time	5 min		5 min	

Table 2: System suitability studies.

Parameter	Cabotegravir	Rilpivirine	Required values
Retention time (minutes)	2.250	2.81	≥ 1.0
Theoretical plate count	4926	8962	>2000
Tailing factor	1.21	1.36	< 2.0
Resolution	NA	4.26	>2
% RSD of peak area	0.9	0.4	< 2.0

Instrumentation

To perform this work, HPLC system (Waters, Milford, MA, USA) of model 2695 alliance coupled with a photodiode array (PDA-type-2996) detector along with Empower 2.0 software was used.

Buffer preparation

Ammonium acetate of about 0.77 g was dissolved in 100 ml of HPLC-grade water to get 0.01 N solution. It was then filtered using 0.22 μm membrane filter and the pH was adjusted to 3.0 using acetic acid.

Mobile phase preparation

A mixture of 0.01 N ammonium acetate and acetonitrile (65:35, v/v) was prepared and sonicated for 5 min before being filtered through a 0.45 μm membrane filter.

Standard stock solution preparation

A stock solution of working standards was prepared by dissolving about 20 mg of cabotegravir and 30 mg of rilpivirine in 50 ml of mobile-phase solvent.

Working standard solution preparation

The standard stock solution was diluted appropriately using mobile phase solvent to get the concentration of 40 $\mu\text{g/ml}$ for cabotegravir and 60 $\mu\text{g/ml}$ for rilpivirine.

Sample solution preparation

One ml of solution from marketed injection formulation (Cabenuva; Label claim 600 mg/3 ml cabotegravir and 900 mg/3 ml of rilpivirine) was diluted using mobile phase solvent to get the sample solution of concentration 40 $\mu\text{g/ml}$ for cabotegravir and 60 $\mu\text{g/ml}$ for rilpivirine and filtered through 0.45 μm membrane filter.

RESULTS AND DISCUSSION

Method development and optimized chromatographic conditions

To get better separation with a proper resolution, tailing factor, and other system suitability parameters, several trials were performed with various ratios of different solvents such as water, acetonitrile, methanol, ammonium acetate on different columns of Kromasil C₁₈ (100 x 4.6 mm, 5 μm), Ace phenyl column (150 x 4.6 mm, 5 μm) and an Inertsil C₁₈ column (150 x 4.6 mm, 5 m). Finally, satisfactory results were obtained by using an Inertsil C₁₈ column (150 x 4.6 mm, 5 m) at a temperature of 30°C with the mobile phase of a mixture of 0.01 N ammonium acetate and acetonitrile (65:35, v/v) pumped at the rate of 1.0 ml/min. The retention times of cabotegravir and rilpivirine were found to be 2.250 min and 2.823 min, respectively when the PDA detector was operated at 257 nm, enabling the shorter run time of 5 min. The optimized chromatogram is shown in Figure 2.

Validation of the proposed method

The developed method was validated as per ICH guidelines Q2 (R1), Validation of analytical procedures: text and methodology.⁹

System suitability

System suitability parameters were assessed using six replicates of the working standard solution of cabotegravir and rilpivirine consisting of 40 µg/ml and 60 µg/ml, respectively. The outcomes were found to be within the limits (Table 2).

Specificity

The method is found to be specific when the chromatograms of the blank, placebo, and working standard solutions showed no interference from dosage form excipients at the retention times of cabotegravir and rilpivirine.

Linearity

Linearity was performed by determining the peak area response at six non-zero concentrations of working standard solutions consisting of 10, 20, 30, 40, 50, and 60 µg/ml of cabotegravir and 15, 30, 45, 60, 75, and 90 µg/ml of rilpivirine. The linearity was observed in the stated concentration range with the regression equation of $y = 4351.x + 1116$, and the regression coefficient of 0.999 for cabotegravir and the regression equation of $y = 5683.x + 1488$ and the regression coefficient of 0.999 for rilpivirine.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

It was done based on the following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times \text{SD}/\text{Slope} \text{ and } \text{LOQ} = 10 \times \text{SD}/\text{Slope}$$

where SD is the standard deviation.

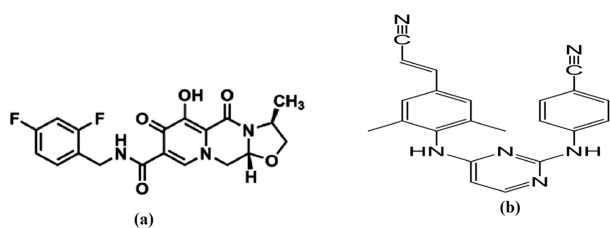


Figure 1: (a) Cabotegravir (b) Rilpivirine.

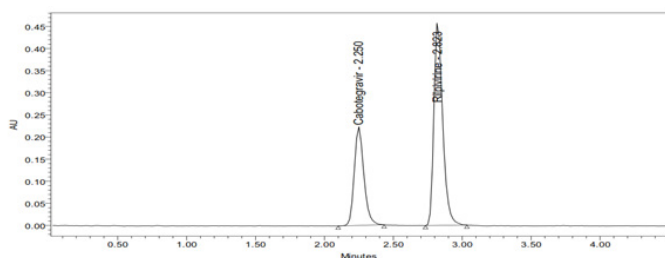


Figure 2: Optimized chromatogram for standard preparation.

The LOD and LOQ were determined to be 0.13 µg/ml and 0.38 µg/ml, respectively for cabotegravir and 0.16 µg/ml and 0.48 µg/ml, respectively for rilpivirine.

Precision

The precision studies were performed at the concentration of 40 µg/ml for cabotegravir and 60 µg/ml for rilpivirine.

Method precision (Repeatability)

The % RSD of six replicate injections of cabotegravir and rilpivirine was assessed to be 0.6% and 0.4% when performed on the same day, demonstrating the precision of the proposed method.

Intermediate precision

Six replicate injections were given on different days and the % RSD values were found to be 0.6% and 0.4%, respectively for cabotegravir and rilpivirine showing that the inter-mediate precision is within the acceptance range.

Accuracy

Recovery tests were performed to validate the method's applicability and accuracy and to look for interferences from the excipients used in the formulation by the standard addition method. The standard solutions of cabotegravir and rilpivirine were prepared at three different concentration levels including 50%, 100%, and 150%. Three determinations were made at each level for recovery and the % mean recovery was found to be in the range of 99.83% to 101.40%, proving the accuracy of the method.

Robustness

The robustness of the method was studied by varying the mobile phase composition ($\pm 5\%$), flow rate (± 0.1 mL/min), and column temperature ($\pm 3^\circ\text{C}$). The %RSD values for the retention times and the peak area of the analytes were then calculated and found to be in the range of 0.09 to 0.36 and 0.20 to 0.90, respectively. It was found to have no significant impact on the retention times and peak area, showing that the method is robust.

Assay for formulation

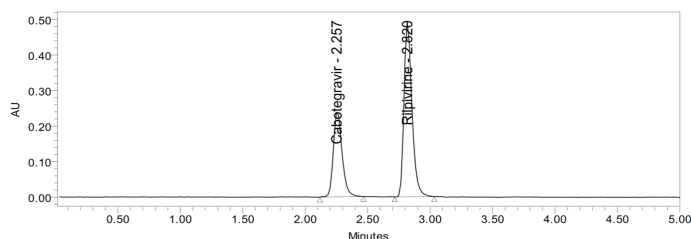
The sample solution was injected into the instrument and the chromatograms were recorded. By application of the proposed method, the % assay of cabotegravir and rilpivirine were found to be 100.06% and 100.27%, respectively. Results are displayed in Table 3. Figure 3 represents the chromatogram of the working sample solution.

Solution stability

The standard solution stability was assessed by injecting it into the instrument after storage for 24 hr at room temperature. It was found to be stable with no change in system suitability parameters.

Table 3: Results of the assay.

Parameter	Cabotegravir	Rilpivirine
Label claim (Brand name - Cabenuva)	200 mg/ml	300 mg/ml
Mean sample peak area	174817	337786
The amount found (mg/ml)	200.12	300.81
% Mean Assay	100.06	100.27
SD	0.61	0.42
% RSD	0.6	0.4

**Figure 3: Chromatogram of working sample solution.****Table 4: Results of forced degradation studies.**

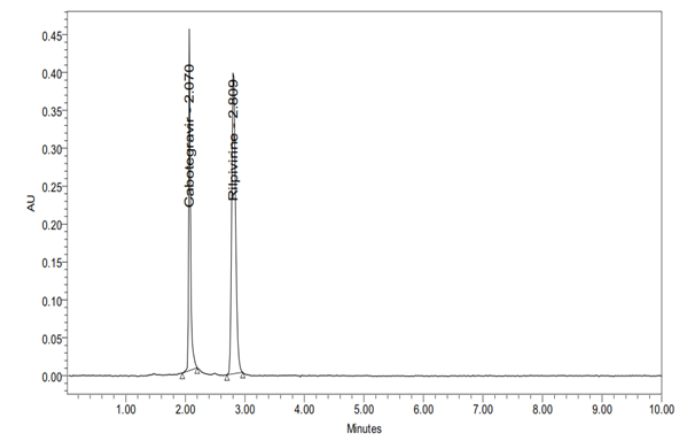
Parameter	Stress conditions	Cabotegravir		Rilpivirine	
		% Recovered	% Degraded	% Recovered	% Degraded
Acidic	Refluxed with 2N HCl for 30 min at 60°C.	96.20	3.80	95.28	4.72
Alkali	Refluxed with 2N NaOH for 30 min at 60°C.	94.36	5.64	94.68	5.32
Peroxide	1mL of 20% hydrogen peroxide at 60°C for 30 min.	93.18	6.82	93.09	6.91
Thermal	Placed in a hot air oven at 105°C for 6 hr.	97.78	2.22	97.82	2.18
Photolytic	Placed in UV chamber for 7 days (200-Watt hours/minutes).	98.54	1.46	98.28	1.72
Neutral (Water)	Refluxed in water for 6 hr at a temperature of 60°C.	99.26	0.74	99.62	0.38

Forced degradation studies

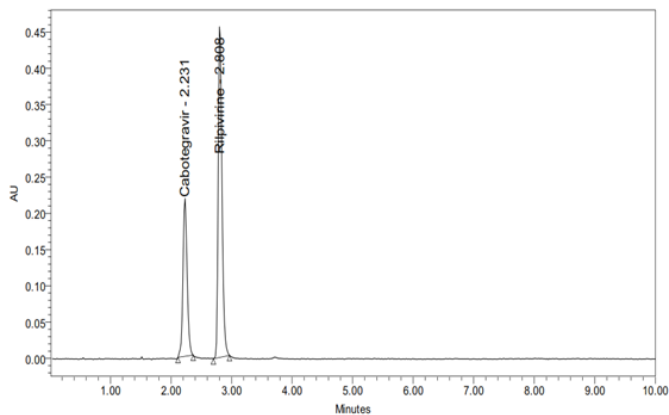
Forced degradation studies for standard solutions were completed with the help of ICH guidelines Q1A (R2), Stability testing of new drug substances and products.¹⁰ Different degradation conditions, including acidic, alkali, oxidative, thermal, photolytic, and neutral, were applied to the standard stock solutions separately. The resulting solutions were subsequently diluted to a working concentration of 40 µg/ml and 60 µg/ml for cabotegravir and rilpivirine, respectively. The solutions were then assessed by the application of the developed method. Though the degradation was observed in all the degradation conditions, the highest degradation was observed with oxidative conditions, and the lowest degradation was seen with neutral conditions. Peaks for the degradation product were observed with alkali and oxidative degradations. Chromatograms for forced degradation studies were shown in Figure 4 and degradation results are depicted in Table 4.

CONCLUSION

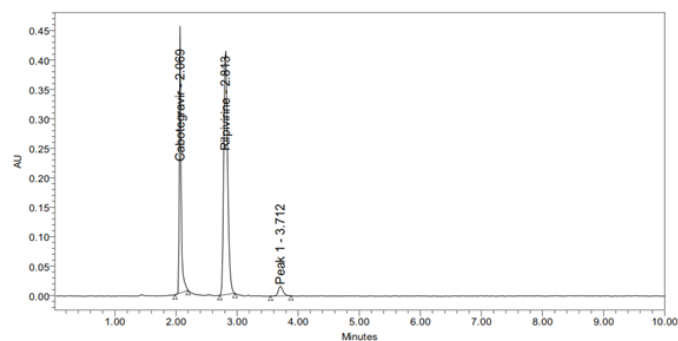
A rapid, specific, sensitive, accurate, precise, and economical RP-HPLC method was successfully developed for the simultaneous estimation of cabotegravir and rilpivirine in bulk and its dosage form. The validation of the method revealed that it is linear, accurate, and unique to the drugs even in the presence of contaminants caused by degradation. The proposed HPLC method is economical as less acetonitrile is required than the reported method. From the LOD and LOQ values, it is observed that the proposed method is significantly more sensitive than the previously reported HPLC method. According to stability results, degradation peaks are discovered in both alkali and oxidative degradation conditions, allowing LC-MS and Fourier Transform Infrared Spectroscopy (FTIR) techniques to use this method for subsequent research. Regular analysis in quality control departments can be performed using the proposed RP-HPLC method.



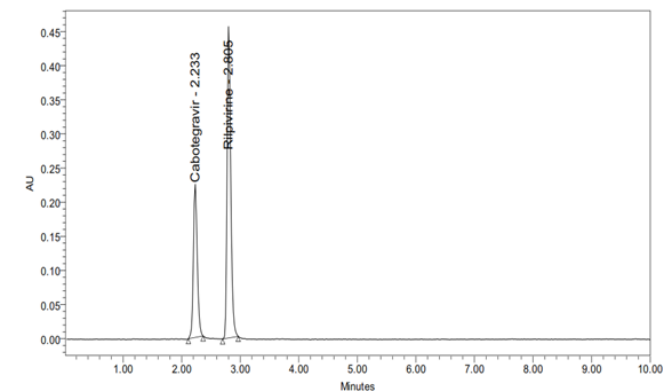
(a)



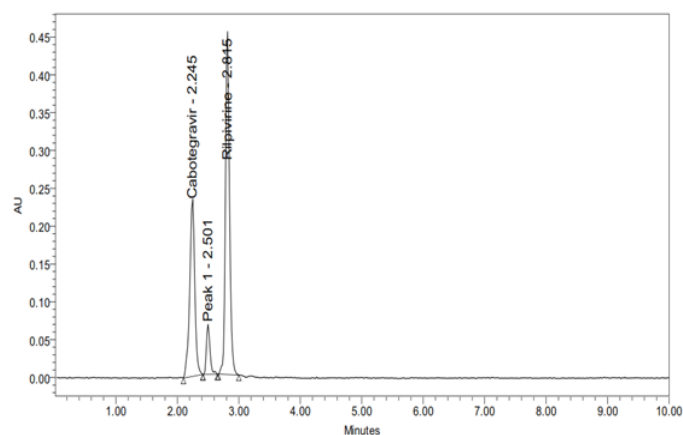
(d)



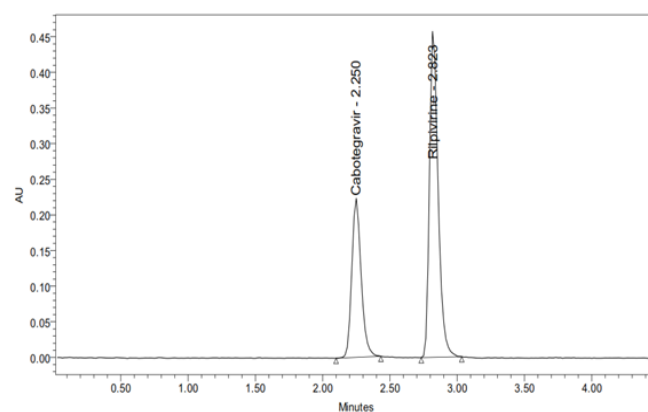
(b)



(e)



(c)



(f)

Figure 4: Chromatograms of forced degradation in (a) acidic conditions (b) alkali conditions (c) oxidative conditions (d) thermal conditions (e) photolytic conditions (f) neutral conditions.

ACKNOWLEDGEMENT

The authors are thankful to Spectrum Pharma Research Solution, Hyderabad, India for providing the API, Anurag University, Hyderabad, India and Anurag Pharmacy College, Kodad, India for providing the necessary facilities for carrying out the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse Phase-High-Performance Liquid Chromatography; **HIV:** Human Immunodeficiency Virus; **API:** Active pharmaceutical ingredients; **RSD:** Relative standard deviation; **SD:** Standard deviation; **mL:** Milliliter; **µg:** Microgram; **%:** Percentage; **v/v:** Volume by volume; **mg:** Milligram; **hr:** Hour; **nm:** Nanometer; **RT:** Retention time; **µm:** Micrometer; **mm:** Millimeter; **min:** Minutes; **NA:** Not Applicable, **PDA:** Photodiode array; **UPLC:** Ultra High-Performance Liquid Chromatography; **UHPLC-MS/MS:** Ultra high-performance liquid chromatography-Tandem mass spectrometry; **HCl:** Hydrochloric acid; **NaOH:** Sodium hydroxide; **LC-MS:** Liquid chromatography-Mass spectrometry; **FTIR:** Fourier transform infrared spectroscopy.

SUMMARY

- Stability indicating RP-HPLC method was developed for simultaneous estimation of cabotegravir and rilpivirine in the bulk and pharmaceutical dosage form.

- The proposed method was found to be economical and very sensitive as compared with the reported method.
- LC-MS and Fourier transform infrared spectroscopy (FTIR) approaches can employ this method for further investigation because stability results show that degradation peaks are found in both alkali and oxidative degradation environments.

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Cite this article: Vemuluri PC, Dodda S. Stability Indicating Reverse Phase-high Performance Liquid Chromatography Method for Simultaneous Estimation of Cabotegravir and Rilpivirine. *Indian J of Pharmaceutical Education and Research*. 2023;57(3s):s766-s771.