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Research Article

Elevating Efficiency: Validated RP-HPLC Method for Concurrent Detection of Cabotegravir and Rilpivirine

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Abstract

Background: Type I HIV can be treated by the combination of these drugs. A novel, precise, and accurate RP-HPLC technique was developed and validated for concurrent assessment of cabotegravir and rilpivirine in pure and medicinal dose form.

Materials and methods: The analyte separation was achieved by Waters 2695 HPLC system that comprised of quaternary pumps and photodiode array detector. Mobile phase was in the ratio of (70:30 v/v) acetonitrile and 0.1N potassium di hydrogen phosphate. Flow rate of 1 ml/min was employed. The detector wavelength was at 257 nm. The run time was 5 min.

Results: The regression equation for cabotegravir was found to be y=7596.9x+1542.1 and for rilpivirine it was y=7517.8x+5409. LOD values for cabotegravir and rilpivirine were observed to be 0.25 µg/ml and 1.79 µg/ml respectively. LOQ values for cabotegravir and rilpivirine were found to be 0.77 µg/ml and 5.44 µg/ml respectively. Conclusion: The proposed method was shown to be exact, accurate, and perfect for usage in QC labs for quantitative analysis of pharmaceutical dosage forms, both single and combined.

Keywords: Cabotegravir; Rilpivirine; C18 column; Validation; Method development

Introduction

Antiretroviral medication cabotegravir is a structural counterpart of dolutegravir. Inhibiting strand transfer of the viral genome into the host genome and stopping virus replication, cabotegravir interacts to the active site of HIV integrase. Due to the daily oral tablet administration and the monthly intramuscular suspension administration, the medication has a protracted period of action. Rilpivirine belongs to the class of compounds known as diary pyrimidines, which are similar to the pyrimidine nucleotides present in DNA. Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used to treat HIV-1 infections in patients who have never received treatment [1]. Figures 1 and 2 represent the chemical structures of cabotegravir and rilpivirine respectively. According to literature survey, it was found that RP-HPLC, LC-MS, UPLC methods were the works performed till date for both drugs in combination [1-9]. The study's major objective was to create an easy, precise RP-HPLC method for the measurement of cabotegravir and rilpivirine in both pure and pharmaceutical dosage forms.

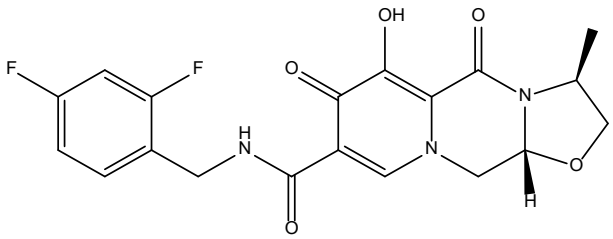

Figure 1: Structure of cabotegravir

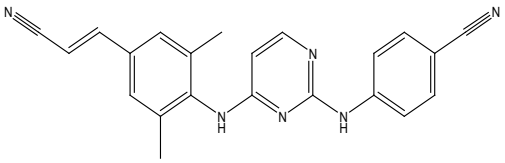

Figure 2: Structure of rilpivirine

Materials and Methods

Chemicals and reagents

Spectrum pharma research solutions (Hyderabad) provided a free sample of rilpivirine and cabotegravir. Ortho-phosphoric acid, potassium dihydrogen ortho phosphate (KH_2PO_4) buffer, acetonitrile and methanol were purchased

from Rankem Laboratories Pvt.Ltd. Millipore Milli Q water was prepared in the laboratory.

Instruments and equipment

Waters 2695 HPLC system equipped with quaternary pumps and photodiode array detector was used. The pH of the solutions was calculated by a pH meter (BVK enterprises, India). All analytical measurements were done on analytical balance (Denver).

Method of analysis

Preparation of standard stock solutions: 37.5 mg of rilpivirine and 25 mg of cabotegravir working standards were accurately weighed and placed into a fifty millilitres clean dry volumetric flask. 10 millilitres of diluents were added and sonicated for ten minutes. Diluents were used to make up the final volume. The obtained standard stock solution was 1000 µg/ml.

Preparation of sample working solutions: A volumetric flask of capacity 10 ml was filled with 100 µg/ml of diluent after 1 ml of each stock solution was pipetted out and added.

Preparation of diluents: Diluent was prepared using acetonitrile and water (50:50 v/v).

Preparation of sample stock solutions: 1 ml of the rilpivirine and cabotegravir injection sample was pipetted into a 100 ml volumetric flask along with 50 ml of diluents. The mixture was then subjected to 25 minutes of sonication. Finally, diluent was added to the volume (1000 µg/ml) and filters were used to remove impurities.

Preparation of sample working solutions: After being filtered, 0.5 ml of the sample stock solution was transferred to a 10 ml volumetric flask and diluted with diluents.

Chromatographic conditions: The RP-HPLC method development and validation of rilpivirine and cabotegravir was carried on Kromasil C18 column. Mobile phase consisted of 0.1N KH_2PO_4: Acetonitrile in the ratio of 70:30 v/v with a flow rate of 1 ml/min. The sample injector volume was 10 µl. Temperature of column was ambient. The wavelength of rilpivirine and cabotegravir from the UV spectrum was 257 nm. Analyte eluted was observed at 257 nm. Table 1 listed the chromatographic conditions, and Figure 3 represented the optimised chromatogram.

Table 1: Optimized chromatographic conditions

Parameter	Chromatographic condition
Stationary phase	Kromasil C18
Mobile phase	0.1N KH_2PO_4: Acetonitrile (70:30)
Column temperature	30°C
Injection volume	10 µl
Run time	5 mins
Flow rate	1 ml/min
λmax	257 nm

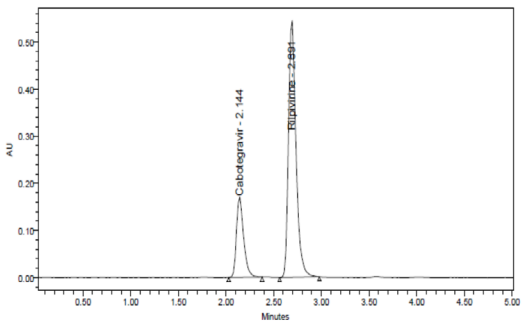

Figure 3: Optimized chromatogram of cabotegravir and rilpivirine

Method development

Trial 1 was carried out by using water: Methanol in the ratio of 50:50. Trial 2 was conducted out by using OPA: Methanol in the ratio of 50:50. Trial 3 was involved out by using acetonitrile: 0.1N KH_2PO_4 in the ratio of 50:50. Trial 4 was included out by using acetonitrile: KH_2PO_4 in the ratio of 75:25. The method optimization was done in the trial 5 by 0.1N KH_2PO_4: Acetonitrile.

Method validation

System suitability parameters: 6 duplicate injections of the drug standard solution of 10µg/ml were injected into HPLC system and the system suitability parameters were determined. Peak tailing, USP theoretical plates count, and the resolution were noted.

Specificity: The specificity was evaluated by loading samples into the HPLC system. Drug solution was compared to the blank solution. The output chromatograms were examined for the interference between a drug peak response and a blank response.

Linearity: Dissimilar drug standard solutions were made to evaluate the linearity by diluting the drug stock solutions with diluents in different concentrations of rilpivirine and cabotegravir ranging from 18.75 µg/ml to 112.5 µg/ml and 12.5 µg/ml to 75 µg/ml respectively. The linearity plot of the calibration curve was assessed by linear regression analysis.

Sensitivity: The following equations, which were based on the slope of the calibration and the standard deviation of the responses using various concentrations of the standard stock solution, were used to calculate the limit of detection and limit of quantification.

Limit of detection=3.3 × standard deviation of the response/ slope of calibration curve of the analyte.

Limit of quantification=10 × standard deviation of the response/slope of calibration curve of the analyte.

Accuracy: Accuracy was determined at 50%, 100% and 150% by adding an acknowledged amount of sample stock solution of rilpivirine and cabotegravir to the standard stock solution. The percent recoveries were calculated.

Precision: Precision was studied as system precision, intraday and inter day. Intraday precision was determined

by injecting 6 different concentrations of standard solutions in the same day. The peak area was measured, and the %RSD was computed. Inter-day precision was measured by injecting 6 different concentrations of standard solutions 3 times a week for 3 days. The peak area was measured, and the %RSD was computed.

Robustness: The determination of the robustness was done by injecting the samples by varying the mobile phase ratio and flow rate.

Forced degradation studies

Oxidation: 0.1 ml of the solution was pipetted out from the standard stock solution of rilpivirine and cabotegravir, 20% of 1 ml H_2O_2 was added to it. The solutions were heated at 60°C for 30 mins in a water bath. The solutions were cooled and brought to room temperature. Diluent was used to make up the volume. Solution of 10 µl was injected into the HPLC system.

Acid degradation: 1 ml of the stock mixture of rilpivirine and cabotegravir was mixed with 1 ml of 2N HCl. Then, it was refluxed for 30 minutes at 600°C. The resultant solution was made up with diluents and 10 µl solution were injected into the system.

Alkali degradation: To 1 ml of stock solution of rilpivirine and cabotegravir, 1 ml of 2N NaOH was added and refluxed for 30 mins at 60°C. To determine the sample's stability, the resulting solution was diluted with diluents. The system was injected with 10 µl, and the chromatograms were obtained.

Thermal degradation: The standard sample solution was heated at 105°C for 6 hours. Diluent was used to prepare the solution. After the system has cooled, 10 µl of the solution was added.

Photolytic degradation: 1500 µg/ml and 1000 µg/ml solution was exposed to UV light by keeping the beaker in UV Chamber for 1 day or 200-Watt hours/m^2 in photo stability chamber and 10 µl were injected into the system.

Results

Method optimization of chromatographic conditions

UV spectroscopic analysis of the drug showed maximum absorbance at 257 nm. An appropriate and precise HPLC technique for analysis of cabotegravir and rilpivirine was employed after several trials with different mobile phases were done. The first trail started with water and methanol in the ratio of 50:50. This trail was not selected as only cabotegravir peak was eluted but not rilpivirine. The 2nd trail was with orthophosphoric acid and methanol in the ratio of 50:50. This trail was not selected as broad peak shape was eluted for cabotegravir. The 3rd trail was with acetonitrile and 0.1N KH_2PO_4 in the ratio of 50:50. This was not selected as both the peaks were eluted in void volume range. The 4th trail was with acetonitrile and KH_2PO_4 in the ratio of 75:25. This was not selected as both peaks were eluted with more elution time. The 5th trail was optimised by 0.1N KH_2PO_4 and acetonitrile in the ratio of 70:30 and the obtained chromatogram was found to be in good shape.

Method validation

System suitability parameters: The parameters for the rilpivirine and cabotegravir revealed that the theoretical plates were >2000 and the tailing factor was <2. Table 2 displayed statistics on system appropriateness.

Table 2: System suitability parameters

S No	Rilpivirine			Cabotegravir			
Inj	RT (min)	USP plate count	Tailing	RT (min)	USP plate count	Tailing	Resolution
1	2.139	3856	1.3	2.688	6162	1.33	3.9
2	2.140	3792	1.3	2.692	6509	1.31	3.9
3	2.143	4261	1.2	2.692	6699	1.31	3.9
4	2.144	4120	1.2	2.694	6560	1.27	3.9
5	2.144	3780	1.3	2.694	6547	1.31	3.9
6	2.144	4163	1.3	2.695	5814	1.37	3.8

Linearity: Six linear concentrations of rilpivirine (18.75 µg/ml-112.5 µg/ml) and cabotegravir (12.5 µg/ml-75 µg/ml) were injected in a duplicate manner. Linearity equations obtained for cabotegravir was y=7596.9x+1542.1 and of rilpivirine was y=7517.8x+5409.4. Correlation coefficient obtained was 0.999 for the 2 drugs. The linearity data was provided in Table 3. Figures 4 and 5 showed the calibration curves of cabotegravir and rilpivirine respectively.

Table 3: Linearity data of cabotegravir and rilpivirine

Cabotegravir		Rilpivirine	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
0	0	0	0
12.5	95544	18.75	146830
25	194002	37.5	284904
37.5	285914	56.25	437029
50	386548	75	575373
62.5	471457	93.75	711564
75	571513	112.5	842305

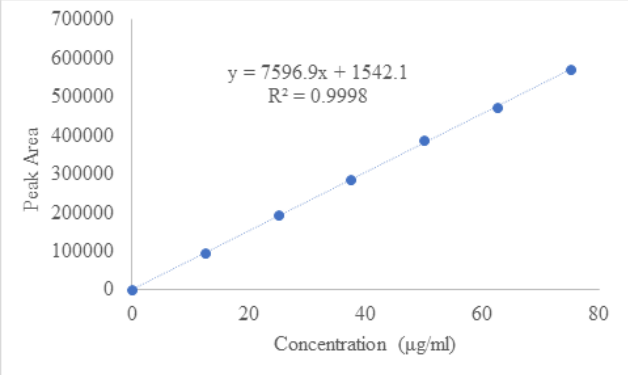

Figure 4: Calibration curve of cabotegravir

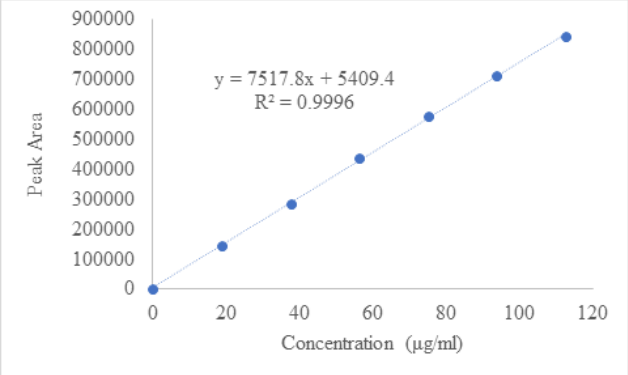

Figure 5: Calibration curve of rilpivirine

Accuracy: Three injections were given for each degree of accuracy. The mean percent recovery for cabotegravir and rilpivirine, respectively, was found to be 100.06% and 99.46%. Tables 4 and 5 showed the accuracy data of cabotegravir and rilpivirine respectively.

Table 4: Accuracy data of Cabotegravir

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %recovery
50%	25	25.03	100.13	100.06%
	25	24.86	99.43	
	25	25.16	100.64	
100%	50	50.28	100.56	
	50	49.66	99.31	
	50	49.66	99.31	
150%	75	74.75	99.67	
	75	75.41	100.55	
		75.68	100.91	

Table 5: Accuracy data of Rilpivirine

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %recovery
50%	37.5	37.215	99.24	99.46%
	37.5	37.216	99.24	
	37.5	37.124	99	
100%	75	75.437	100.58	
	75	74.381	99.17	
	75	74.776	99.7	
150%	112.5	111.518	99.13	
	112.5	111.569	99.17	
	112.5	112.394	99.91	

Repeatability: Average area, SD, and percent RSD were calculated for rilpivirine and cabotegravir. They were found to have respective values of 0.6% and 0.7%. Data on repeatability are displayed in Table 6.

Table 6: Repeatability of Cabotegravir and Rilpivirine

S. No	Area of Rilpivirine	Area of Cabotegravir	112.5
1	575550	381174	112.5
2	574180	384892	112.5
3	579531	388092	112.5
4	571415	382926	112.5
5	579234	383125	112.5
6	572585	386929	112.5
Mean	575416	384523	112.5
S.D	3378.7	2622.3	112.5
%RSD	0.6	0.7	112.5

Intermediate precision: Chromatogram values for intermediate precision were found to be 0.4 and 1.2 for rilpivirine and cabotegravir respectively. The outcomes were shown in Table 7.

Table 7: Intermediate precision of Cabotegravir and Rilpivirine

S. No	Area of Rlpivirine	Area of Cabotegravir	112.5
1	576855	382019	112.5
2	578200	387307	112.5
3	573546	389288	112.5
4	579618	379754	112.5
5	576488	387700	112.5
6	575064	381254	112.5
Mean	576629	384554	112.5
S.D	2164.6	4006.1	112.5
%RSD	0.4	1	112.5

Robustness: The %RSD of flow+, flow-, mobile phase+, mobile phase-, temperature+ and temperature-were found to be 0.4%, 1.1%, 0, 0.9%, 0.4%, 1% respectively for cabotegravir and 0.4, 0.3, 0.5, 0.4, 0.4, 0.4 for rilpivirine respectively. Table 8 provided an illustration of the outcomes.

Table 8: Robustness data of Cabotegravir and Rilpivirine

S.no	Condition	% RSD of Cabotegravir	% RSD of Rilpivirine
1	Flow rate (-) 0.9ml/min	0.4	0.4
2	Flow rate (+) 1.1ml/min	1.1	0.3
3	Mobile phase (-) 65B:35A	0	0.5
4	Mobile phase (+) 75B:25A	0.9	0.4
5	Temperature (-) 27°C	0.4	0.4
6	Temperature (+) 33°C	1	0.4

Specificity: Interference was not detected. The specificity data was shown in Table 9. The chromatogram of blank was shown in Figure 6.

Table 9: Specificity

S. No	Sample details	Retention time (min)
1	Blank solution	Interference is not detected
2	Cabotegravir	2.144 min
3	Rilpivirine	2.692 min

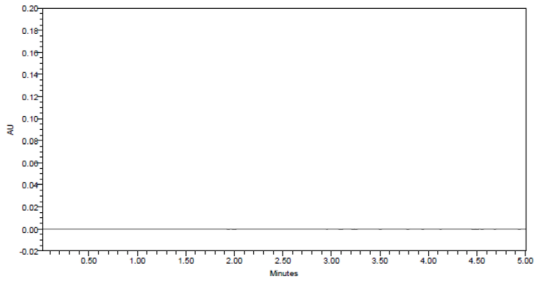

Figure 6: Chromatogram of blank sample

Sensitivity: Cabotegravir and rilpivirine LOD was found to be 0.25 µg/ml and 1.79 µg/ml respectively and cabotegravir and rilpivirine LOQ was found to be 0.77 µg/ml and 5.44 µg/ml.

Assay: Average %assay for rilpivirine and cabotegravir obtained was 99.93% and 99.88% respectively.

Forced degradation studies: Cabotegravir and rilpivirine were subjected to acid degradation (2.65%, 2.53%), base degradation (2.58%, 1.85%), peroxide degradation (4.42%, 4.77%), thermal degradation (2.63%, 2.67%), UV (1.38%, 1.27%), water (0.69%, 0.78%). Table 10 represented the forced degradation studies data. Figures 7-12 listed the chromatograms for the many types of degradation.

Table 10: Forced degradation studies data of Cabotegravir and Rilpivirine

Type of degradation	Rilpivirine			Cabotegravir		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	561265	97.47	2.53	374768	97.35	2.65
Base	565178	98.15	1.85	375038	97.42	2.58
Peroxide	548383	95.23	4.77	367966	95.58	4.42
Thermal	560461	97.33	2.67	374851	97.37	2.63
UV	568539	98.73	1.27	379659	98.62	1.38
Water	571364	99.22	0.78	382300	99.31	0.69

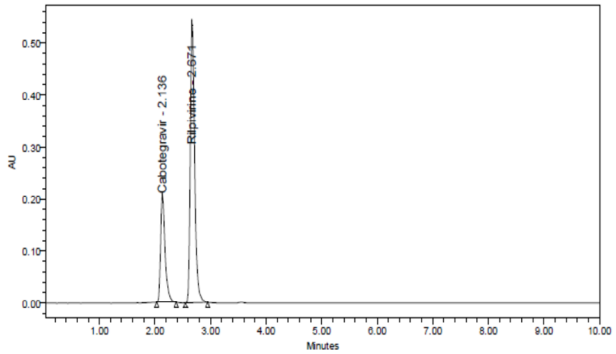

Figure 7: Chromatogram of acidic degradation of cabotegravir and rilpivirine

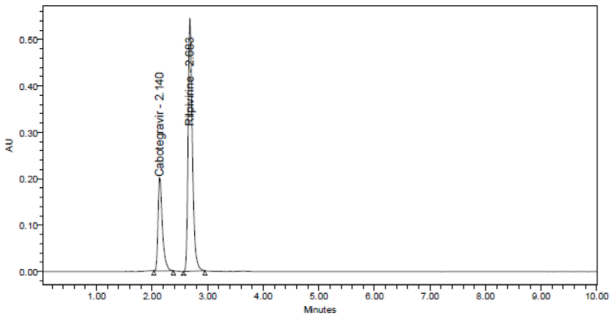

Figure 8: Chromatogram of alkali degradation of cabotegravir and rilpivirine

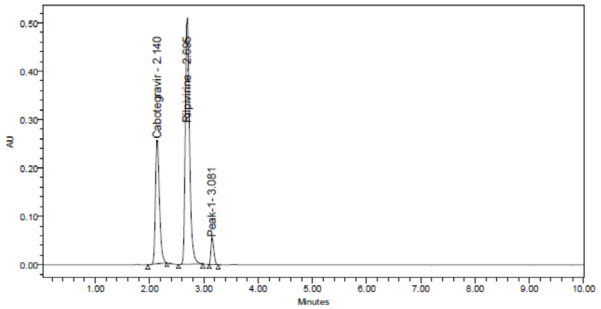

Figure 9: Chromatogram of peroxide degradation cabotegravir and rilpivirine

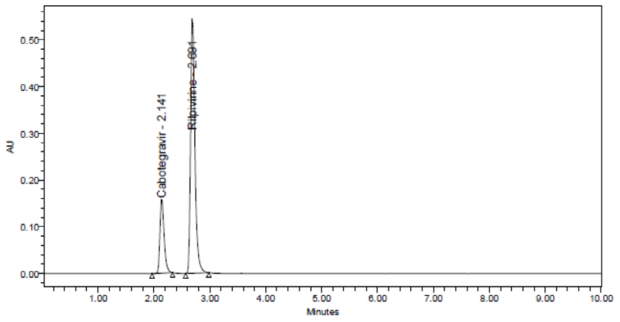

Figure 10: Chromatogram of thermal degradation cabotegravir and rilpivirine

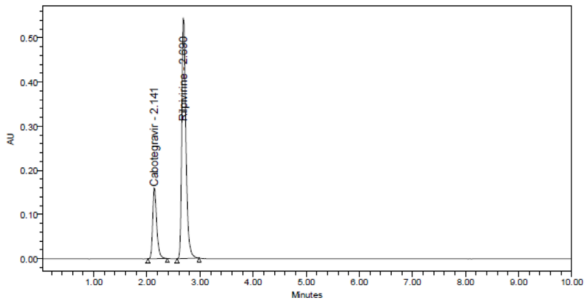

Figure 11: Chromatogram of UV degradation cabotegravir and rilpivirine

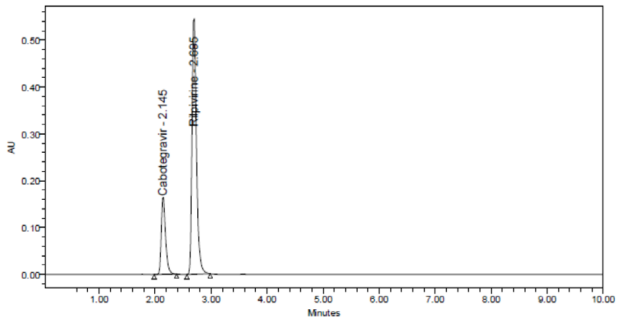

Figure 12: Chromatogram of water degradation cabotegravir and rilpivirine

Discussion

RP-HPLC technique for the quantification of cabotegravir and rilpivirine in the pure and tablet dosage forms was developed and validated as per ICH guidelines. The analyte separation was obtained by Kromasil C18 column. Mobile phase was in the ratio (70:30 v/v) of 0.1N of KH_2PO_4 and acetonitrile Flow rate of 1 ml/min was used. The wavelength of the drug was at 257 nm. The development of the method required a system suitability test that ensured the system was appropriate for the analysis of cabotegravir and rilpivirine. A proper protocol was established to ensure that the HPLC equipment executed techniques that produced findings with an acceptable level of accuracy and precision prior to the analysis of samples from each day. With the proposed HPLC approach, linearity was attained at a concentration range of 18.75 µg/ml-112.5 µg/ml for rilpivirine and 12.5 µg/ml-75 µg/ml for cabotegravir. When the correlation coefficient was found to be within accepted limits, acceptable linearity was indicated. The conventional addition technique was used to obtain accuracy samples at three levels (50%, 100%, and 150%). The method's high recovery rates demonstrated that the suggested approach can be used for quality control examination. The repeatability of the chromatograms was found to be within the predetermined limit (%RSD not more than 2.0%). As a result, it proved that the method was found to be repeatable. The limit specified (%RSD not more than 2.0%) for chromatogram data with intermediate precision was determined to be met. Thus, it demonstrated that the procedure was determined to be effective. The robustness was evaluated by introducing small, deliberate changes to the chromatographic conditions, which include the ratios of 0.1N KH_2PO_4 and acetonitrile in the mobile phase (65B:35A) and (75B:25A), and the flow rate of the mobile phase (0.9 ml/min and 1.1 ml/min). The %RSD was discovered to be robust.

Specificity was examined by introducing a blank solution into the HPLC apparatus. This showed that there was no interference in the blank sample at the retention time of the standard cabotegravir and rilpivirine sample. At the retention time of the typical cabotegravir and rilpivirine, there was no interference with the blank sample. So, the procedure was specific, as can be seen. LOD was defined as about S/N 3 and LOQ as the minimal verified concentration with (%) RSD and (%) error 20%, all of which were taken into consideration when calculating LOD and LOQ. LOD and LOQ were observed to be sensitive. As per the label claim, the amount of drug content obtained from the sample solutions values was in the permissible 90%-110% range. Cabotegravir and rilpivirine were subjected to acid degradation (2.65%, 2.53%), base degradation (2.58%, 1.85%), peroxide degradation (4.42%, 4.77%), thermal degradation (2.63%, 2.67%), UV (1.38%, 1.27%), water (0.69%, 0.78%). The results were less than 10% indicating that cabotegravir and rilpivirine were more resistant towards all forced degradation conditions applied. The system suitability parameters were also within limits. Table 11 represents the summary of the approach that was created and verified.

Table 11: Summary

Parameters	Rilpivirine		Cabotegravir	LIMIT
Linearity Range (µg/ml)	18.75 µg/ml-112.5 µg/ml		12.5 µg/ml-75 µg/ml	-
Regression coefficient	0.999		0.999	-
Slope (m)	7517.8		7517.8	-
Intercept (c)	5409.4		5409.4	-
Regression equation (Y=mx+c)	y=7517.8x+5409.4		y=7517.8x+5409.4	R<1
Assay (%mean assay)	99.93%		99.88%	90%-110%
Specificity	Specific		Specific	No interference of any peak
System precision %RSD	0.7		1.2	NMT 2.0%
Method precision %RSD	0.6		0.7	NMT 2.0%
Accuracy %recovery	99.46%		100.06%	98%-102%
LOD	1.79		0.25	NMT 3
LOQ	5.44		0.77	NMT 10
Robustness	FM	0.4	0.4	-
	FP	1.1	0.3	%RSD NMT
	MM	0	0.5	2
	MP	0.9	0.4	
	TM	0.4	0.4	
	TP	1	0.4	

Conclusion

The proposed method was authenticated for a various parameter, included accuracy, precision, linearity, specificity, system suitability, and robustness, as specified by ICH requirements. The results attained fit the criteria for approval. The method can therefore be used successfully for the routine analysis of cabotegravir and rilpivirine in bulk and pharmaceutical dose forms as it is easy to use, precise, inexpensive and safe.

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Conflict of Interest

Regarding the research and authorship of this publication, the authors state that there are no conflicts of interest.

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