THE DEVELOPMENT OF A NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS EVALUATION OF RILPIVIRINE AND CABOTEGRAVIR IN PURE API FORM AND TABLET DOSAGE IN ACCORDANCE WITH ICH GUIDELINES

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Abstract: This study's focus is on the simultaneous assessment of Rilpivirine and Cabotegravir using RP-HPLC in bulk and tablet dosage form. **Materials and methods:** The separation was carried out on a Zorbax SB C18 (4.6 x 150mm, 5 m) analytical column using a mobile phase of 40% Water (0.1 percent Formic Acid): 60% Acetonitrile. Using a UV detector, the eluents were found at 248.0 nm. **Results:** Under optimal circumstances, Rilpivirine and Cabotegravir were separated at 2.084 and 3.2mins, respectively. The detection limit for Rilpivirine was 1.02µg/mL, while the detection limit for Cabotegravir was 3.30µg/mL. Cabotegravir had a percentage mean recovery of 100.02 percent, but Rilpivirine had a recovery rate of 100.72 percent. **Conclusion:** The percentage of degradation was determined to be extremely low in each stressful environment. It was found that optimized conditions were incredibly ideal for simultaneously determining all of them in both marketing dose form and bulk form.

Keywords: Cabotegravir, Rilpivirine, method development, validation, and RP-HPLC.

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INTRODUCTION

A subclass of non-nucleoside reverse transcriptase inhibitors includes Rilpivirine (NNRTIs). A group of HIV integrase inhibitors includes Cabotegravir. [(1)] The chemical formula for Rilpivirine is 4-[4-(4-[(E)-2-cyanovinyl] -2, 6-dimethylphenyl} amino) pyrimidine-2-yl]amino}benzonitrile. Chemically, Cabotegravir is N-[(2, 4-Difluorophenyl) methyl]. -6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11a-hexahydro(1,3)oxazolo(3,2-a)pyrido(1,2-d)pyrazine-8-carboxamide. For the treatment of human immune deficiency virus type 1 (HIV-1) infection in some individuals, Rilpivirine and Cabotegravir injections are combined. [(2–5)]These drugs function by lowering the blood level of HIV. Although Rilpivirine and Cabotegravir do not treat HIV, they may lessen your risk of getting AIDS and other HIV-related conditions such serious infections or cancer. [(6–8)] Taking these medications, engaging in safer sexual behaviour, and changing other aspects of one's lifestyle may help reduce the risk of spreading the HIV virus to others. Figures 1 and 2 depict the structures of Rilpivirine and Cabotegravir, respectively. [(9-11)] By enhancing sensitivity and shortening elution periods, the current research aims to create an ultrafast, stability-indicating RP-HPLC method for the simultaneous quantification of Rilpivirine and Cabotegravir in bulk pharmaceuticals and commercial dosage forms. Additionally, the validation research was completed in accordance with ICH Guidelines Q2 (International Conference on Harmonization) (R1).

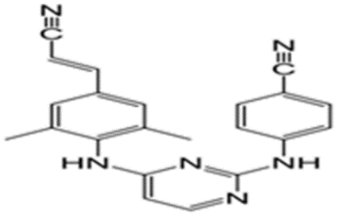

Figure 1. Chemical Structure of Rilpivirine

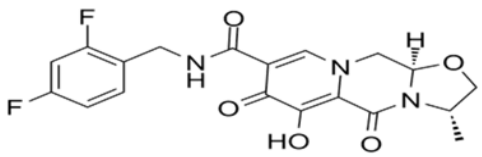

Figure 2. Chemical Structure of Cabotegravir

MATERIALS AND METHODS

Chemicals:
Mylan Labs R&D Division, Hyderabad, generously contributed pharmaceutical-grade Rilpivirine and Cabotegravir. FINER Chemical LTD, LICHROSOLV (MERCK), and Sigma Aldrich (Mumbai) provided analytical reagent grade solvents and chemicals for this study.
Instruments:
Afcoset ER-100A analytical balance, WATERS, software: Empower, 2695 separation module, UV detector, LABINDIA UV 12.500, Adwa – AD 10100 pH meter, Borosil Pipettes, Burettes and Beakers.

HPLC method development:
Mobile phase preparation:
Accurately measured 600 ml (60 percent) of Acetonitrile HPLC and 400 ml (40 percent) of water containing 0.1 percent Formic acid were combined and degassed in an ultrasonic water bath for 10 minutes before being filtered through a 0.45 µ filter under vacuum filtration.
Preparation of the Diluent: The Mobile phase served as the diluent.

Rilpivirine and cabotegravir standard and sample solution preparation:
Preparation of standard Solution:
30 mg of Rilpivirine and 20 mg of Cabotegravir working standards should be accurately weighed and transferred into a 25 ml clean, dry volumetric flask. Add around 7 mL of diluent, sonicate the mixture to completely dissolve it, and then add more liquid to the desired amount using the same solvent. (Stock solution)
Pipette 3 ml of the aforementioned stock solutions into a 10 ml volumetric flask and diluent until the desired concentration is reached.

Preparation of sample Solution:
Take a precise sample of liquid solution containing 30 mg of Rilpivirine and 20 mg of Cabotegravir, transfer it into a 25 mL clean, dry volumetric flask, add about 7 mL of diluent, and sonicate the mixture for up to 15 minutes to completely dissolve it. Then, add enough liquid to the flask to reach the desired volume using the same solvent. The 0.45 micron Injection filter is then used to filter it. (Stock solution)
Pipette 3 ml of Rilpivirine and Cabotegravir from the aforementioned stock solution into a 10 ml volumetric flask, and then add diluent until the desired concentration is reached.
Pipette 3 ml of Rilpivirine and Cabotegravir from the aforementioned stock solution into a 10 ml volumetric flask, and then add diluent until the desired concentration is reached.
Procedure:
The procedure is to inject 20µl of the standard, place the sample into the chromatographic system, measure the areas of the Rilpivirine and Cabotegravir peaks, and then use the formulas to calculate the assay's percentage.

Mobile Phase Optimization:
The first mobile phases tested were acetonitrile: methanol, methanol: orthophosphoric acid buffer, methanol: phosphate buffer, and acetonitrile: methanol with different pH combinations and different ratios. The mobile phase was then adjusted to contain acetonitrile in a 40:60 v/v ratio and phosphate buffer (pH 3.0).

Wavelength selection:
The UV spectrum of rilpivirine and cabotegravir at 10µg/ml in diluents (the composition of the mobile phase) was captured by scanning in the 1000–400 nm range. 248 nm was chosen as the wavelength from the UV spectrum. At this wavelength, both pharmaceuticals exhibit strong absorption.

UV Graph

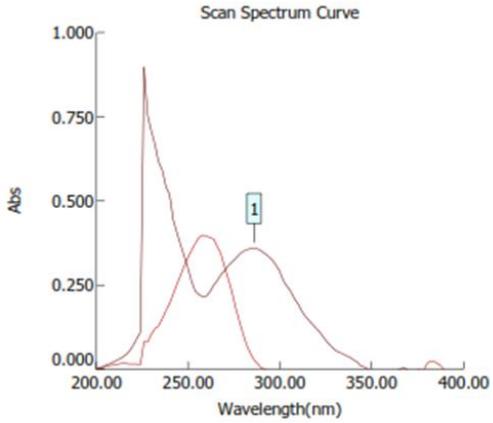

Figure 3. Isobestic point of Rilpivirine and Cabotegravir

Optimization of Colum:
The best column discovered to be Xterra RP18 (4.6 x 150mm, 5µm), which provided good peak shape and resolution at 1.0 ml/min.
Optimized chromatographic conditions:
Instrument used : Waters HPLC with auto sampler and UV detector.
Temperature : Ambient
Column : Zorbax SB C 18 (4.6 x 150mm, 5µm)
Mobile phase : 40% Water (0.1% Formic Acid): 60% Acetonitrile
Flow rate : 1 ml per min
Wavelength : 248 nm
Injection volume : 20 µl
Run time : 10 min.

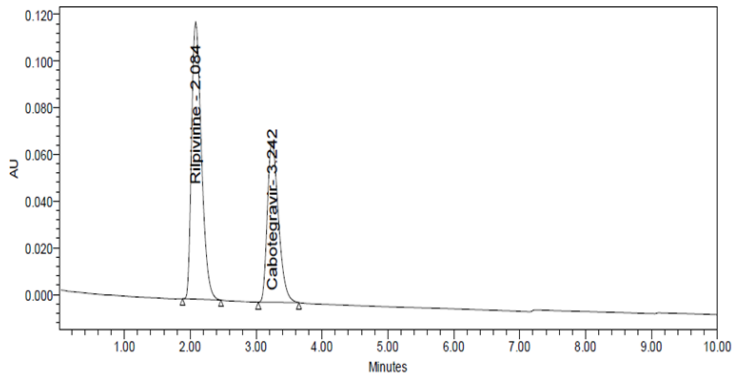

Figure 4. Optimized RP-HPLC Chromatogram of Rilpivirine and Cabotegravir

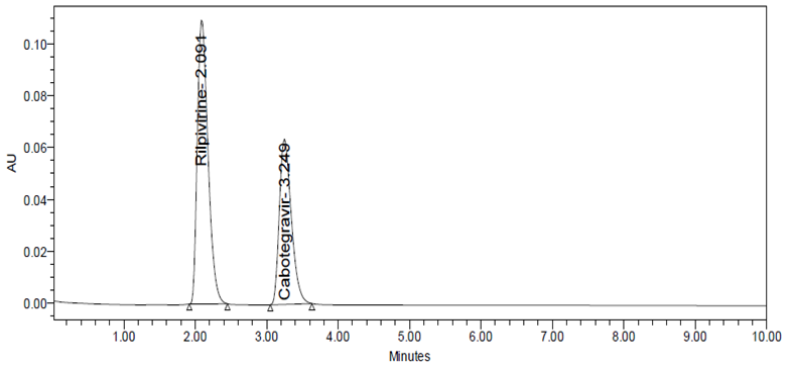

Figure 5. RP-HPLC Chromatogram of marketed Rilpivirine and Cabotegravir

RESULTS

Method validation:

System suitability study

It was done to ensure that an analytical system was functioning properly. It was tested by injecting Rilpivirine (0.36 mg/mL) and Cabotegravir (0.24 mg/mL) six times each in to the chromatographic system. The calculation of percentage relative standard has taken into account a number of factors, including theoretical plate, retention duration, and asymmetry factor.

Specificity:

Blank and standard are injected into the chromatographic system for specificity. With regard to the retention times of the analytical peaks, there is no interference from any peak in the blank.

Precision:

From the primary standard stock solution, a spiked solution containing 800 µg/mL of Cabotegravir and 1200 µg/mL of Rilpivirine was made for the precision study of the current approach. Six injections of the produced solution were made, and the area of each injection was measured using HPLC. The area of six replicate injections' percent RSD was found to be within the predetermined bounds. The area of results from six standard injections should not have an RSD of more than 2%.

Table 1: Results of Precision study

Injection	Area for Cabotegravir	Area for Rilpivirine
Injection-1	111368	852828
Injection-2	112717	852337
Injection-3	112655	858355
Injection-4	113939	852839
Injection-5	1112.513	858513
Injection-6	112282	857582
Average	112662.3	855409.0
Standard Deviation	845.7	12.524.5
%RSD	0.8	0.4

Intermediate precision:

For this investigation, the spiked solution including all four APIs was prepared according to precision study guidelines and was injected on a different day six times, with the area of each injection being assessed in HPLC. The area of results from six

standard injections should not have %RSD of more than 2%. Table 2: Results of Intermediate precision study

Table 2: Results of Intermediate precision study

Injection	Area for Rilpivirine	Area for Cabotegravir
Injection-1	859453	112535
Injection-2	857162	111224
Injection-3	859458	112915
Injection-4	858377	113391
Injection-5	858482	113108
Injection-6	859771	112959
Average	858783.8	112688.7
Standard Deviation	976.1	769.7
%RSD	0.1	0.7

Accuracy study:

A recovery study was used to look at the precision of the newly developed technique. Standard samples of Cabotegravir and Rilpivirine were added to a fixed-dosage tablet powder mixture at concentrations of 50%, 100%, and 150%, respectively. Calculations were done to figure out how much each dosage yielded overall. For each level, the percentage of RSD was calculated, and the overall recovery for all medications was analyzed. Each step should have a recovery rate between 98.0 and 102.0 percent.

Table 3: Accuracy study results of Rilpivirine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	678142.3	15	15.10	100.40	100.72
100%	1361348	30	30.39	100.78	
150%	2045898	45	45.73	100.97	

Table 4: Accuracy study results of Cabotegravir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	432916.3	10	10.55	100.43	100.02
100%	861056.3	20	19.97	99.88	
150%	1289755	30	29.40	99.74	

Linearity

Different concentrations of Rilpivirine and Cabotegravir have been generated from the standard solution with the appropriate dilution in order to examine the linearity of the currently established approach. For Rilpivirine 120-600 µg/mL, Cabotegravir 80-4000 µg/mL solutions were prepared at different levels and injected for the linearity study. All of the drugs calibration curves were plotted at various levels. The linearity graph was built with concentration and peak area in mind, and the data that resulted was put through a regression analysis.

Table 5: Linearity Results of Rilpivirine

S. No	Linearity Level	Concentration	Area
1	I	120	463750
2	II	240	913263
3	III	360	1343348
4	IV	480	1799353
5	V	600	2210576
Correlation Coefficient			0.999

Table 6: Linearity Results of Cabotegravir

S. No	Linearity Level	Concentration	Area
1	I	80	299387
2	II	160	595567
3	III	240	867190
4	IV	320	1165070
5	V	400	1459454
Correlation Coefficient			0.999

Limit of detection study (LOD)

A working standard solution containing 30 mg of Rilpivirine and 20 mg of Cabotegravir was carefully weighted and diluted as previously reported in order to examine the limit of detection. By taking an adequate quantity of aliquote volume out of the aforementioned solution, a further dilution was created to create 1.02 µg/mL of Rilpivirine and 0.81 µg/mL of Cabotegravir. S/N ratio, which needs to be 3, has been calculated taking into account baseline noise and signal noise.

Table 7: LOD Results of Rilpivirine and Cabotegravir

S.NO	Peak name	Area	Height	USP Resolution	USP Tailing	USP Plate count
1.	Rilpivirine	1598	152		1.4	2914
2.	Cabotegravir	1369	151	3.8	1.3	3775

Limit of quantitation (LOQ) study

Sample solutions for the quantitation limit experiment were created using the working standard solution presented earlier in this section. Then, by dilution of appropriate quantities of the standard solution with the diluent in a 10mL volumetric flask, we created solutions of Rilpivirine 3.30 µg/mL/mL and Cabotegravir 2.67 µg/mL/mL. The produced solutions underwent analysis using the chromatographic apparatus. S/N ratio, which needs to be 10, has been calculated taking into account baseline noise and signal noise.

Table 8: LOQ Results of Rilpivirine and Cabotegravir

S.NO	Peak name	Area	Height	USP Resolution	USP Tailing	USP Plate count
1.	Rilpivirine	3587	598		1.4	2918
2.	Cabotegravir	2672	618	3.7	1.3	3786

Robustness

Several optimized parameters, including the flow rate of the mobile phase, the organic makeup of the mobile phase, and the detection wavelength of the optimized conditions, were purposefully altered at a low level in the robustness analysis of the currently proposed method. The organic component changed to 10%, and the flow rate was altered by a factor of 0.1. To observe the robustness, the tailing factor and theoretical plate were taken into account. The parameters' percentage relative standard deviation was determined, and it shouldn't be higher than 2.

Table 9: System suitability results for Rilpivirine (Change in flow rate)

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2885	1.43
2	1.0	2913.10	1.40
3	1.1	2915	1.39

Table 10: System suitability results for Cabotegravir (Change in flow rate)

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	3736	1.39	3.94
2	1.0	3772.39	1.37	3.96
3	1.1	3751	1.35	3.96

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 11: System suitability results for Rilpivirine (Change in composition of mobile phase):

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2922	1.43
2	*Actual	2913.10	1.40
3	10% more	2927	1.38

Table 12: System suitability results for Cabotegravir (Change in composition of mobile phase):

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3290	1.34	5.70
2	*Actual	3772.39	1.37	3.96
3	10% more	3554	1.38	2.95

*Results for actual Mobile phase composition (40:60) Water: Acetonitrile has been considered from Accuracy standard

Forced degradation study

The current sample solution underwent a force degradation investigation employing the ICH-recommended stress conditions of acidic, alkaline, oxidative, thermal, and photolytic stress.

Each sort of degradation study has been carried out three times, and the computation has taken into account the mean peak area.

Hydrolytic degradation under acidic condition

Pipette A 10 ml volumetric flask was filled with 3 ml of the aforementioned solution and 3 ml of 0.1N HCl. The volumetric flask was then maintained at 60 °C for 24 hours, neutralised with 0.1 N NaOH, and diluted to 10 ml. Place the filtered solution in vials using 0.44 micron syringe filters.

Hydrolytic degradation under alkaline condition

Add 3 ml of the aforementioned solution and 3 ml of 0.1N NaOH to a 10 ml volumetric flask using a pipette. The volumetric flask was then maintained at 60°C for 24 hours, neutralised with 0.1N HCl, and diluted to a final volume of 10ml. Place the filtered solution in vials using 0.44 micron syringe filters.

Thermal induced degradation

A sample of Cabotegravir and Rilpivirine was collected in a petridish and maintained in a hot air oven at 110^0 C for three hours. Following sample collection and dilution with diluents, the HPLC was used to analyse the material.

Oxidative degradation

A 10 ml volumetric flask was filled to the proper level with diluent after adding 3 ml of the stock solution and 1 ml of hydrogen peroxide (12.5 percent w/v) in it. After that, for 15 minutes, the volumetric flask was left at room temperature. Place the filtered solution in vials using 0.45 micron syringe filters.

Photo degradation

Pipette 3 ml of the stock solution or more into a 10 ml volumetric flask and place it in the sun for 24 hours. The volume was then diluted to the required amount. Place the filtered solution in vials using 0.45 micron syringe filters.

Table 13: Stability study Results

Sample Name	Rilpivirine	
	Area	% Degraded
Standard	1348112	
Acid	1257384	6.7
Base	1317780	2.3
Peroxide	1279224	5.1
Thermal	1282324	4.9
Photo	1290818	4.3
Sample Name	Cabotegravir	
	Area	% Degraded
Standard	860373	
Acid	823807.1	4.3
Base	801953.7	6.8
Peroxide	837831.2	2.6
Thermal	830862.2	3.4
Photo	838691.6	2.5

DISCUSSION

The goal of the current work was to develop a reverse phase (RP)-HPLC approach that was straightforward, diplomatic, accurate, and precise for the analysis of Rilpivirine and Cabotegravir in both their pure and pharmaceutical dose forms. Rilpivirine and Cabotegravir were found to have holding durations of 2.084 and 3.2 minutes, respectively. Each standard was injected five times to obtain consistent peak regions for each standard. There is a strong link between the discussions and the area under the curves (r=0.999). The reports revealed precision, and the percent RSD value was shown to be below 3.00, indicating that the suggested HPLC procedure was precise and accurate. Tables 3 and 4 show how much drug was found to have been recovered. The results of the investigation showed that the proposed method was reliable, with only minor variations in the composition of the mobile phase, flow rate, and temperature. The proposed RP-HPLC technique was therefore designed to be quick, simple, precise, accurate, and time-efficient.

CONCLUSION

The simultaneous detection of Rilpivirine and Cabotegravir using RP-HPLC was found to be very similar, based on empirical data. It was determined that the novel procedure was far superior to the previously described methods in every manner. For simultaneous analysis in bulk form and approved dosage form, all of the tested APIs were determined to be relevant and resolute under optimal conditions.

Compliance and Ethical Standards

Ethical Approval: NA

Funding details: NA

Conflict of interest: No

Informed consent: NA

Author's Contribution:

Each author contributed to the conception, design and execution of the study and also agreed to submit to the current journal.

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