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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Cabotegravir and Rilpivirine in Pharmaceutical Dosage Form

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ABSTRACT

For the simultaneous estimation of Rilpivirine and Cabotegravir in pharmaceutical dosage form, a simple, accurate, and precise method was created. The analytical chromatographic technique was run through column Agilent 150 (4.6 x 150mm, 5µm). Mobile phase containing Acetonitrile: OPA taken in the ratio 54:46 was pumped through column at a flow rate of 1 ml/min. Buffer used is Opa adjusted ph 2.4 with adding 0.1% Trifluroacetic acid. Temperature was maintained at 30°C. Optimized wavelength selected was 257 nm. Retention time of Rilpivirine and Cabotegravir were found to be 2.271 min and 2.723. %RSD of the Rilpivirine and Cabotegravir were and found to be 0.7 and 0.8 respectively. %Recovery was obtained as 100.45% and 100.11% for Rilpivirine and Cabotegravir respectively. LOD, LOQ values obtained from regression equations of Rilpivirine and Cabotegravir were 0.12, 0.37 and 0.07, 0.20 respectively. Regression equation of Cabotegravir is y = 32637x + 5878, and y = = 32637x + 5878 of Rilpivirine.

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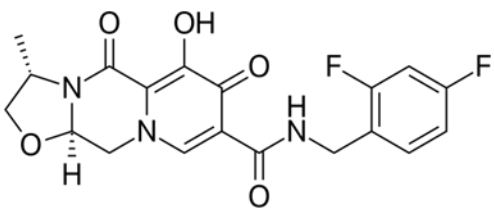
INTRODUCTION:

The HIV-1 pandemic is indisputably the most significant public health crisis of our time. It is a complex mash-up of various epidemics that exist both within and between different nations and regions of the world [1]. These estimates fail to capture the dynamic nature of this epidemic as it changes over time and evolves in terms of magnitude, viral diversity, geographic distribution, and mode of transmission. No part of the world is currently unaffected by this pandemic [2]. Although combination antiretroviral therapy can significantly increase a person's life expectancy, human immunodeficiency virus (HIV) infection is still a chronic condition that requires daily oral medication for the rest of a person's life [3,4,14].

Cabotegravir/Rilpivirine is a combination drug used for the treatment of HIV/AIDS, As an alternative, two-drug regimens have been developed [5,6], Cabenuva, is a co-packaged antiretroviral medication approved by the FDA for treatment of Antiretroviral [7], FDA-approved, once-monthly injection of the full HIV treatment regimen for adults [8], In the US, co-packaged medication has received medical approval[9], For the treatment of HIV-1 infection in adults and adolescents 12 years of age and older weighing at least 35 kg, intramuscular extended-release cabotegravir and rilpivirine are recommended as a full regimen to replace the current antiretroviral regimen[10], Inhibiting strand transfer of the viral genome into the host genome and stopping virus replication, cabotegravir binds to the active site of HIV integrase. A non-competitive NNRTI called Rilpivirine binds to reverse transcriptase. HIV-1 replication and other RNA and DNA-dependent DNA polymerase activities are inhibited as a result of its binding. It does not exhibit activity against the following human DNA polymerases [11,12,13],

There are some other RP-HPLC methods published [14,15,16].

Structure of Cabotegravir



Structure of Rilpivirine

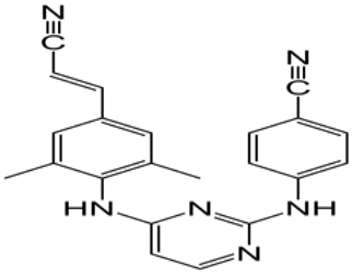


Figure No. 1: Structures of Cabotegravir and Estetrol

Several analytical approaches have been described; however, according to a thorough assessment of the literature 14, 15, 16, no method for stability indicating estimation has been reported. As a result, a simple, cost-effective stability-indicating simultaneous estimate in pharmaceutical dosage form by RP-HPLC in pharmaceutical dosage form must be developed and validated in accordance with ICH (Q2 standard)[17].

MATERIALS AND REAGENTS

Pure medications Rilpivirine and Cabotegravir were delivered by Akrivis Pharma Private Limited of Hyderabad. A local pharmacy provided the Rilpivirine and Cabotegravir (Cabenuva) combination Injection. All of the chemicals and buffers used in this method were given by Rankem in India.

INSTRUMENTATION

WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector with software Empower 2.

CHROMATOGRAPHIC CONDITIONS:

Flow rate:	1ml/min
Column:	Agilent C18 150x 4.6mm, 2.7µ.
Mobile phase:	0.1% OPA: Acetonitrile (54:46)
Detector:	240.0 nm
Temperature:	Ambient
Injection volume:	10.0µL
Run time:	10.0 mins

PREPARATION OF SOLUTIONS

Preparation of 0.01N Potassium dihydrogen phosphate Buffer: Accurately weighed 1.36gm of Potassium dihydrogen ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with

water then added 1ml of Triethylamine then PH adjusted to 4.0 with dil. Orthophosphoric acid solution.

Preparation of Standard solution: In a 50 ml clean, dry volumetric flask, accurately weigh and transfer 30 mg of rilpivirine and 20 mg of cabotegravir from working standards. Add ¾th of diluent, sonicate for 10 minutes, and add diluents to the final volume as needed. (400 mg of Cabotegravir and 600 mg of Rilpivirine)

Standard Working solution:

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml Rilpivirine of and 40µg/ml of Cabotegravir)

Preparation of Sample solution: Pipette out 1ml of Rilpivirine and Cabotegravir injection sample into a 100 volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by filters. (3000µg/ml Rilpivirine of and 2000µg/ml of Cabotegravir).

Sample working solution: 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml Rilpivirine of and 40µg/ml of Cabotegravir)

METHOD VALIDATION

The validation of the HPLC method was carried out in accordance with the ICH recommendations for the simultaneous estimation of Rilpivirine and Cabotegravir drug material to show that the method is suitable for routine analysis.

System suitability:

The system suitability parameters were determined by preparing standard solutions of Rilpivirine (60ppm) and Cabotegravir (40ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. System suitability chromatogram was shown in figure 2 and values are mentioned in the table 1.

Specificity (Selectivity): Checking for interference in the method that was optimized. At the retention times of these drugs using this method, we shouldn't observe interfering peaks in the placebo or blank groups. This method was therefore described as specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2.

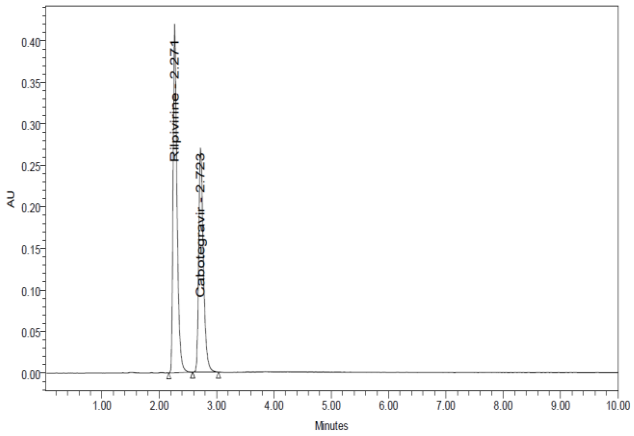


Fig. No. 2- Optimized Chromotogram

RESULT- Rilpivirine and Cabotegravir were eluted at 2.271 min and 2.732 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Table No. 1: System suitability results

S no	Rilpivirine			Cabotegravir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.263	5155	1.39	2.717	5235	1.35	3.2
2	2.268	5280	1.37	2.730	5723	1.33	3.3
3	2.272	5245	1.38	2.732	5312	1.33	3.3
4	2.273	5335	1.42	2.739	5825	1.39	3.4
5	2.277	5252	1.45	2.747	5503	1.35	3.3
6	2.286	5483	1.38	2.765	5680	1.34	3.4

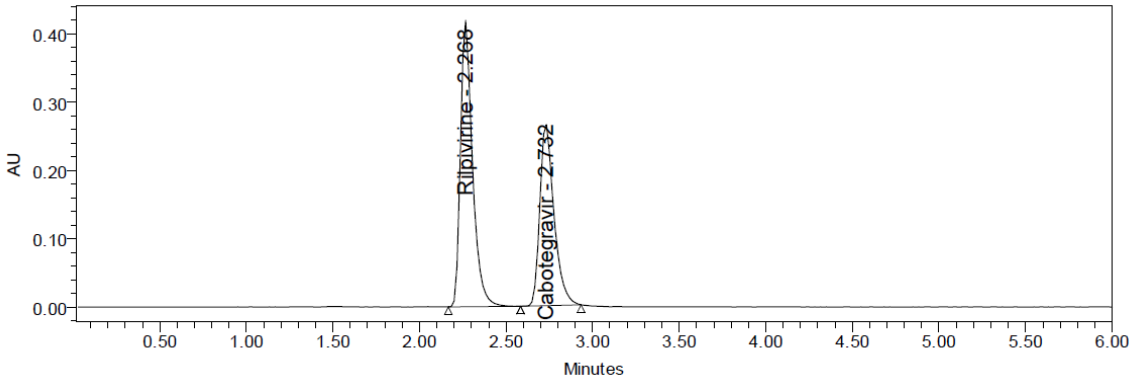
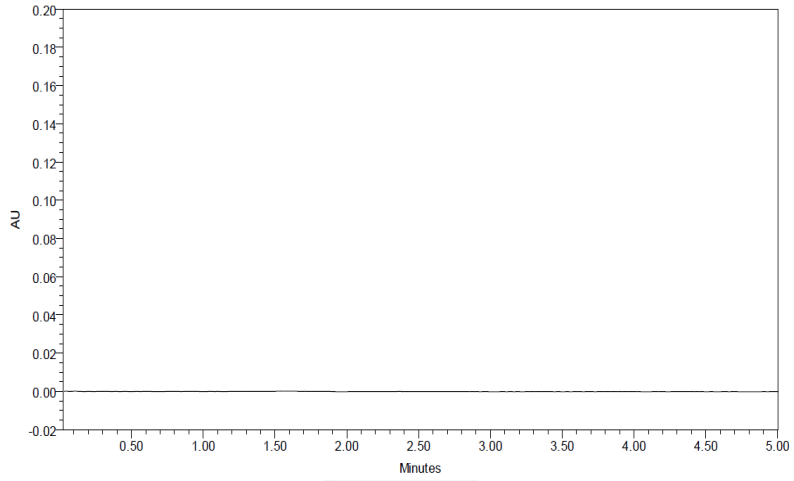


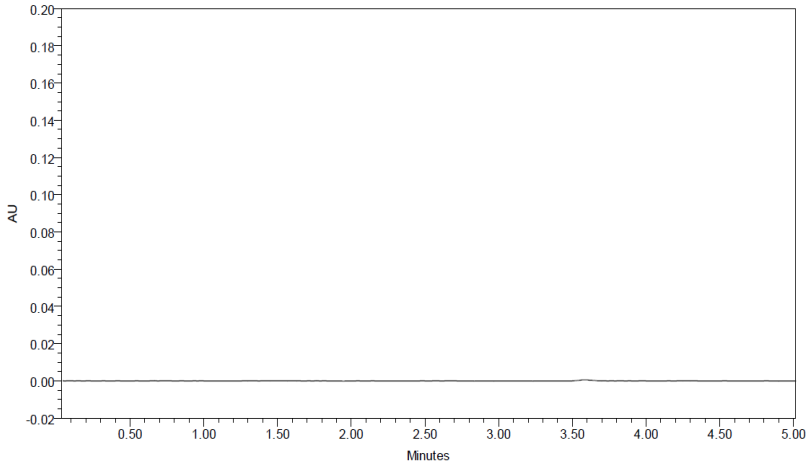
Figure No. 3: System suitability Chromatogram of Rilpivirine and Cabotegravir.

Table No. 2: Specificity data

Sample name	Retention time (mins)
Rilpivirine	2.268
Cabotegravir	2.732



Blank Chromatogram



Placebo Chromatogram

Figure No. 4: Specificity Chromatograms of Rilpivirine and Cabotegravir.

Table No. 3: Linearity

Six linear concentrations of Cabotegravir (0-60ml/ µg/ml)) and Rilpivirine (0-90µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Cabotegravir was y = 38940x + 2460 and of Rilpivirine was y = 32637x + 5878. Correlation coefficient obtained was 0.999 for the two drugs.

Cabotegravir		Rilpivirine	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
10	392904	15	498209
20	781086	30	994192
30	1174154	45	1492249
40	1545944	60	1927014
50	1977469	75	2459516
60	2323074	90	2950763

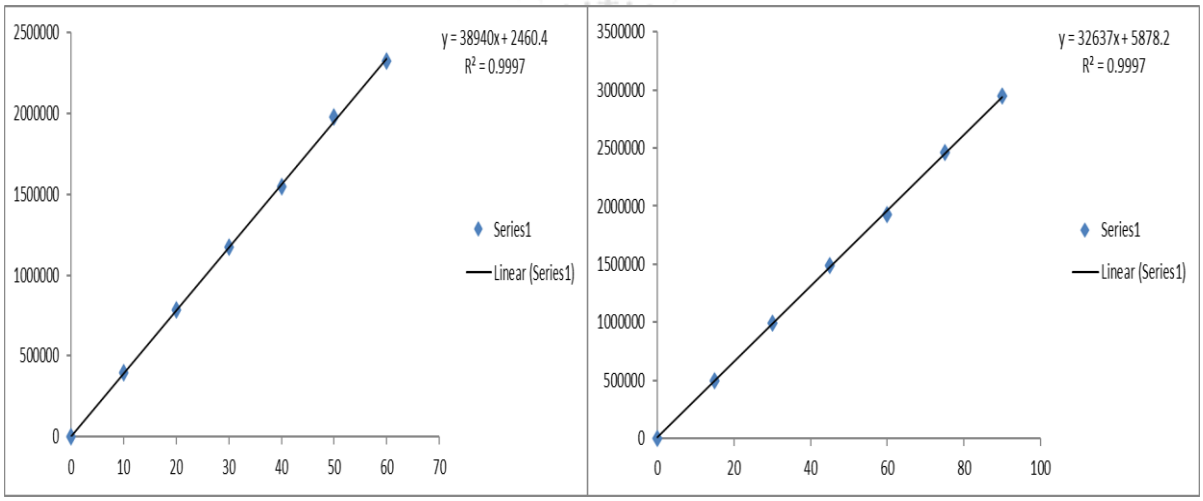


Figure No. 5: Calibration curves of cabotegravir and Rilpivirine

Table No. 4: Accuracy (% Recovery data)

% Level	**% Recovery**					
	Rilpivirine			**Cabotegravir**		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
50% Level	30	29.8	99.2	20	20.08	100.41
	30	29.7	99.0	20	20.14	100.70
	30	30.2	100.5	20	19.81	99.07
100% Level	60	60.0	100.0	40	39.70	99.25
	60	59.8	99.6	40	39.71	99.29
	60	60.2	100.3	40	39.85	99.62
150% Level	90	89.9	99.9	60	60.35	100.58
	90	89.9	99.8	60	60.13	100.22
	90	90.2	100.2	60	60.06	100.09
Mean%			**99.85**			**99.91**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Rilpivirine and Cabotegravir. Results of peak area are summarized in Table 5.

Table No. 5: System precision data

Inj	**Rilpivirine**	**Cabotegravir**
1	1971290	1543233
2	1962036	1577250
3	1970195	1566837
4	1997476	1558690
5	1983876	1562278
6	1962913	1572883
Avg	1974631	1563529
Std dev	13668.6	12030.1
% RSD	0.7	0.8

The % RSD for the peak areas of Rilpivirine and Cabotegravir obtained from six replicate injections of standard solution was within the limit.

Method Precision: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.6% respectively for Rilpivirine and Cabotegravir. As the limit of Precision was less than "2" the system precision was passed in this method. Data obtained is summarized in Table 6.

Table No. 6: Method precision data

Injection	Rilpivirine	Cabotegravir
1	1987484	1579230
2	1998332	1574918
3	1986421	1560854
4	1991308	1555589
5	1975263	1572611
6	1974615	1557408
Avg	1985571	1566768
Std dev	9235.0	10034.1
% RSD	0.5	0.6

From the above results, the % RSD of method precision study was within the limit for Rilpivirine and Cabotegravir.

Sensitivity

Molecule	LOD	LOQ
Cabotegravir	0.07	0.20
Rilpivirine	0.12	0.37

Table No. 7: Robustness results

Chromatographic condition	Rilpivirine (RSD)	Cabotegravir (RSD)
Flow rate (-) 0.9ml/min	0.6	1.1
Flow rate (+) 1.1ml/min	0.4	0.8
Mobile phase (-) 65B:35A	0.4	1.1
Mobile phase (+) 55B:45A	0.9	1.0
Temperature (-) 27°C	0.7	0.4
Temperature (+) 33°C	0.5	0.4

Table No. 8: Forced degradation conditions for Rilpivirine and Cabotegravir.

Stress condition	Solvent	Temp (^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxdation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table No. 9: Degradation profile results

Type of degradation	Rilpivirine		Cabotegravir	
	% RECOVERED	% DEGRADED	% RECOVERED	% DEGRADED
Acid	97.78	2.22	95.18	4.82
Base	95.69	4.31	95.81	4.19
Peroxide	93.14	6.86	94.46	5.54
Thermal	97.50	2.50	97.89	2.11
Uv	98.19	1.81	98.54	1.46
Water	99.01	0.99	99.04	0.96

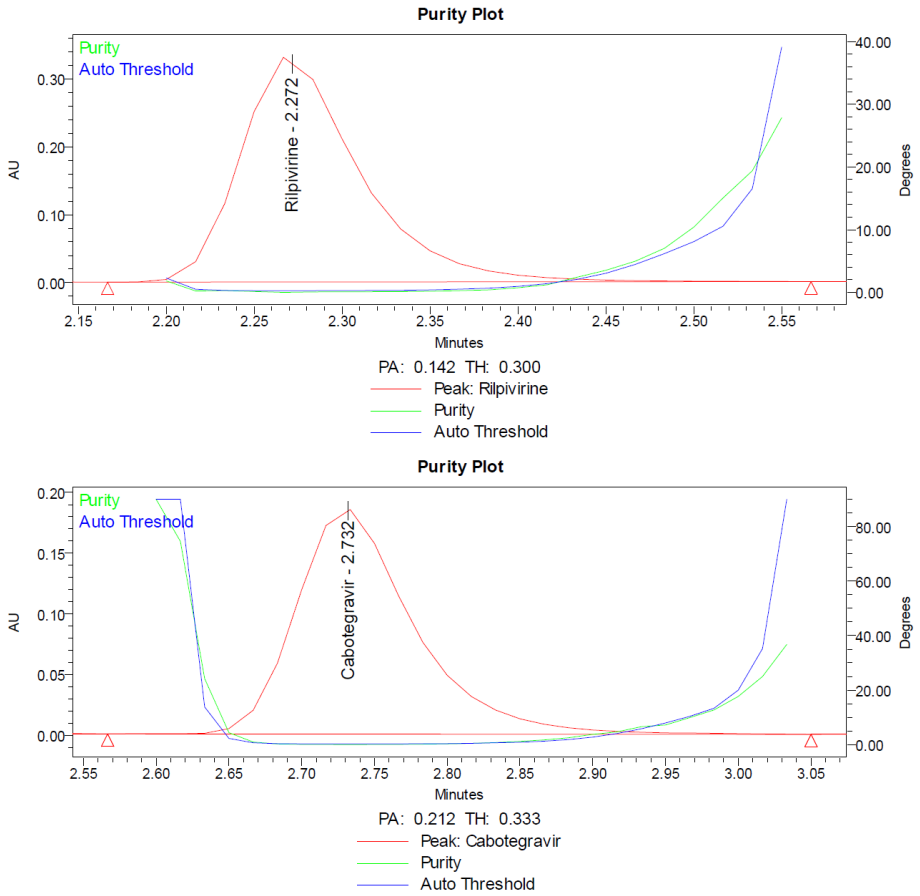


Fig. No 6: Degradation purity plots

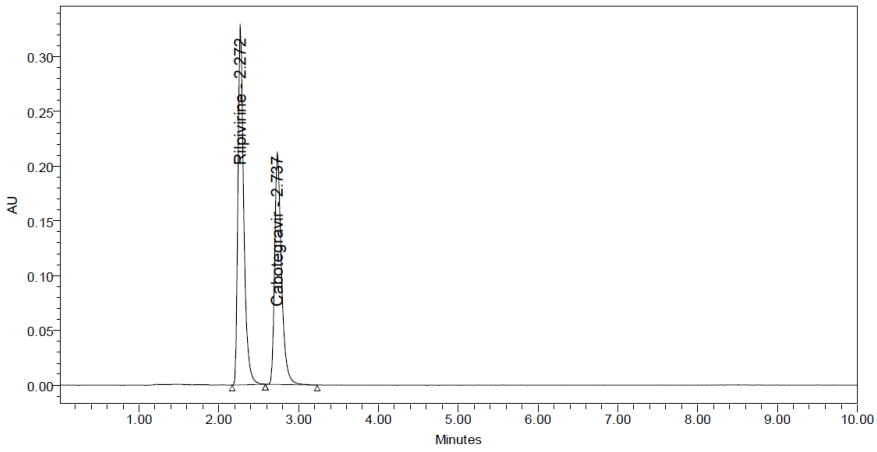


Fig. No. 7: Acid degradation chromatogram

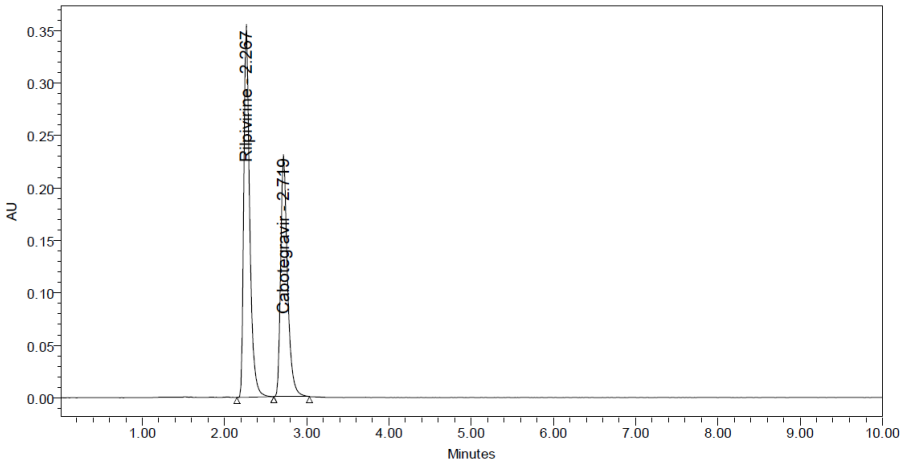


Fig. No. 8: Base degradation chromatogram

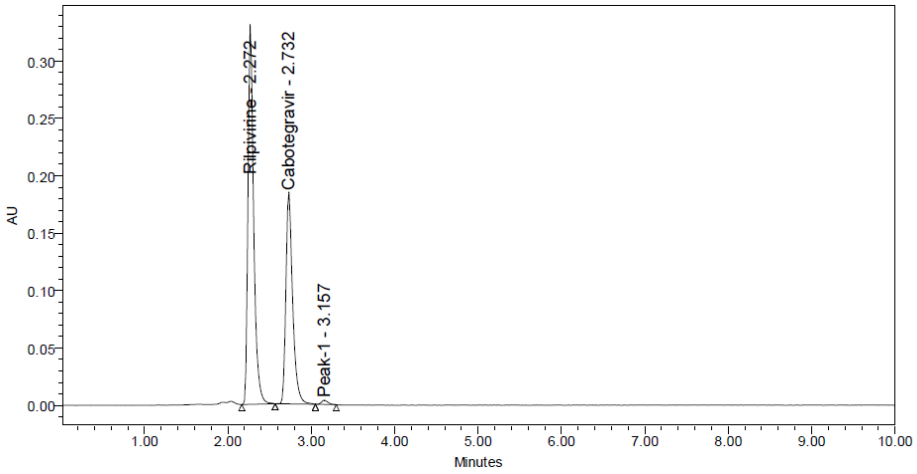


Fig. No. 9: Peroxide degradation chromatogram

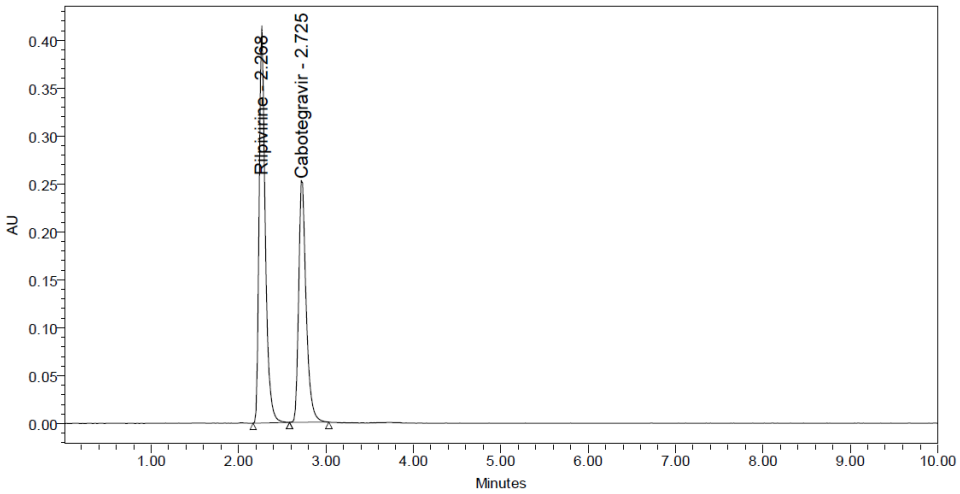


Fig. No. 10: Thermal degradation chromatogram

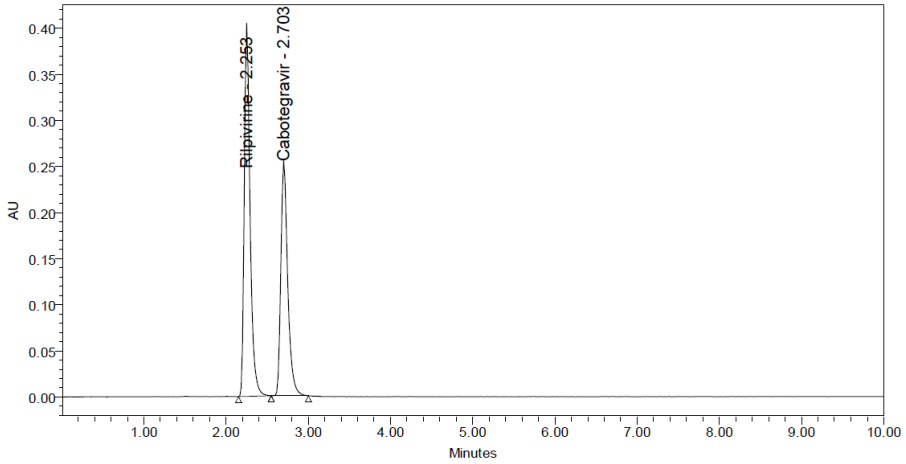


Fig. No. 11: UV degradation chromatogram

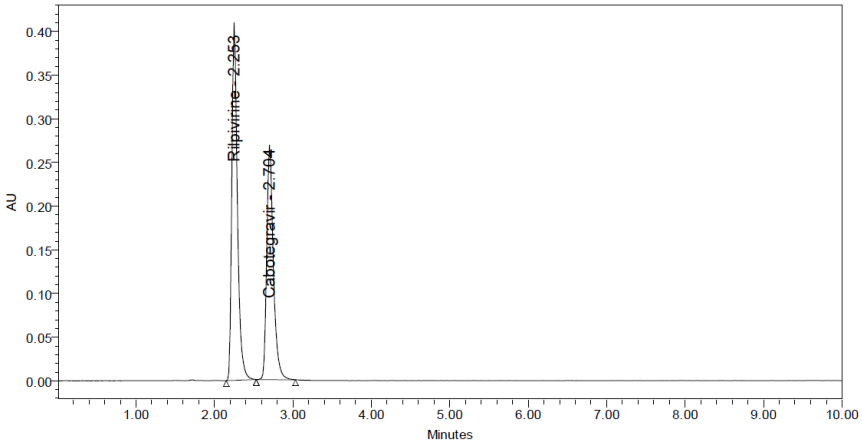


Fig. No. 12: Water degradation chromatogram

Table No. 10: Assay results for Rilpivirine and Cabotegravir

Drug name	Label claim dose	%Assay	Brand Cabenuva
Rilpivirine	300mg/1ml	100.45%	
Cabotegravir	200mg/1ml	100.11%	

CONCLUSION

The RP-HPLC methodology was used to create and evaluate a new stability indicating analytical approach. The sample preparation is straightforward, uses less mobile phase, and takes very little time to analyze. The results of the study will be highly beneficial for quality monitoring of Rilpivirine and Cabotegravir in pharmaceutical dosage forms. The assay examination of two medications from a combination dosage form using this devised method yielded results that were nearly 100 % accurate. The results of the recovery studies were good, indicating that there was no interference from excipients.

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