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Research Article

Method Development and Validation of Simultaneous Estimation of Cabotegravir and Rilpivirine Using RP-HPLC Method

L.Swathi,[1] G. Anusha Reddy,[2] Dr. K. Atchuta Kumar[3]

[1]Assoc.Professor, Srinivasa Rao College of Pharmacy, P.M Palem, Vishakapatnam.
[2]M.Pharmacy, Department of Pharmaceutical Analysis and Quality Assurance, Srinivasa Rao College of Pharmacy, P.M Palem, Affiliated to Andhra University, Vishakapatnam - 530041, Andhra Pradesh, India.
[3]Professor, Srinivasa Rao College of Pharmacy, P.M Palem, Vishakapatnam.

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ABSTRACT

For the simultaneous estimate of Cabotegravir and Rilpivirine in pharmaceutical dose form, a simple, accurate, and exact approach was established. BDS C8 150 x 4.6 mm, 5m chromatogram was done. A mobile phase containing 0.1% orthophosphoric acid:acetonitrile in a 50:50 ratio was pushed down the column at a flow rate of 1.0 ml/min. 0.1% Ortho phosphoric acid buffer was utilized in this procedure. The temperature was kept at 30°C. The optimal wavelength chosen was 257 nm. Cabotegravir and Rilpivirine retention times were determined to be 2.950 min and 3.518.%RSD of Cabotegravir and Rilpivirine were found to be 0.8 and 0.5, respectively. %Cabotegravir and Rilpivirine recovery rates were 99.19% and 98.90%, respectively. LOD and LOQ values derived from regression

Keywords: Cabotegravir, Rilpivirine, RP-HPLC

INTRODUCTION

HIV is a sexually transmitted infection (STI). It can also be spread by contact with infected blood and from illicit injection drug use or sharing needles. It can also be spread from mother to child during pregnancy, childbirth or breastfeeding. Without medication, it may take years before HIV weakens your immune system to the point that you have AIDS. There's no cure for HIV/AIDS, but medications can control the infection and prevent progression of the disease. Antiviral treatments for HIV have reduced AIDS deaths around the world, and international organizations are working to increase the availability of prevention measures and treatment in resource-poor countries. [1]

Cabotegravir and Rilpivirine are the drugs, which are used for the treatment of HIV-1.

Cabotegravir : Cabotegravir is an HIV-1 integrase inhibitor used for treatment and pre-exposure prophylaxis of HIV-1 infection.

Cabotegravir, or GSK1265744, is an HIV-1 integrase inhibitor that is prescribed with the non-nucleoside reverse transcriptase inhibitor, rilpivirine.[2,3,4] Early research into cabotegravir showed it had lower oral bioavailability than dolutegravir,[4] which resulted in the development of long acting monthly intramuscular injection formulation for cabotegravir.[2,4]

Address for Correspondence: L. Swathi, Assoc. Professor, Srinivasa Rao College of Pharmacy, P.M Palem, Vishakapatnam; **E-Mail:** anu220947@gmail.com

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Cabotegravir was granted FDA approval on 21 January 2021 in combination with rilpivirine to treat HIV-1 infection in virologically suppressed individuals.[5]

Rilpivirine: Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with other antiretrovirals to specifically treat human immunodeficiency virus type 1 (HIV-1).

Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients.[6] It is a diarylpyrimidine derivative[7] The internal conformational flexibility of rilpivirine and the plasticity of it interacting binding site gives it a very high potency and reduces the chance of resistance compared to other NNRTI's.8 Rilpivirine was developed by Tilbotec, Inc. and FDA approved on May 20, 2011.[9]

Cabotegravir and rilpivirine combination injection are used together for the treatment of the human immunodeficiency virus type 1 (HIV-1) infection. HIV is the virus that causes acquired immune deficiency syndrome (AIDS). This medicine is usually given to patients to replace their current anti-HIV medicines when their healthcare provider determines that they meet certain requirements.

Cabotegravir and rilpivirine combination injection will not cure or prevent HIV infection or AIDS. It helps keep HIV from reproducing and appears to slow down the destruction of the immune system. This may help delay problems that are usually related to AIDS or HIV disease from occurring. This medicine will not keep you from spreading HIV to other people. People who receive this medicine may continue to have other problems usually related to AIDS or HIV disease.[10]

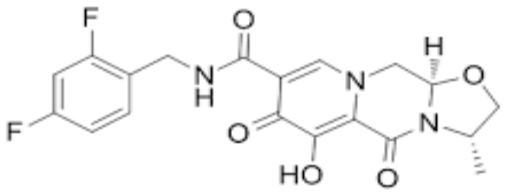

Figure 1. Structure of Cabotegravir

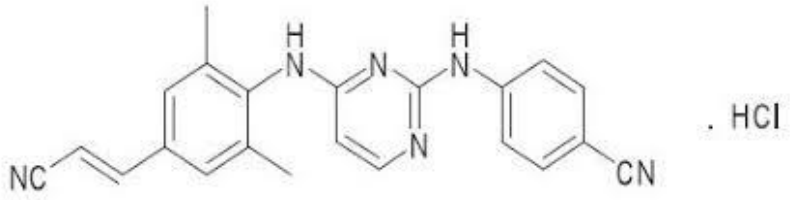

Figure 2. Structure of Rilpivirine

According to a literature review, there are some techniques for the simultaneous estimate of these medicines as well as others for assessment of the drugs alone or in combination with other drugs. Utilizing UV-Spectrophotometry RP-HPLC. There is no established technique for the stability-indicating simultaneous measurement of Cabotegravir and Rilpivirine by RP-HPLC in pharmaceutical dosage form, according to a survey of the literature. The primary goal of this work is to provide an efficient, quick, and accurate RP-HPLC approach for estimating of Cabotegravir and Rilpivirine in medicinal dose and tablet form. According to ICH recommendations, a proven approach was also used to estimate the amounts of Cabotegravir and Rilpivirine.[11-13]

MATERIALS AND REAGENTS

Cabotegravir and Rilpivirine pure drugs were received from Spectrum Pharma research solutions, Hyderabad. The combination tablet Cabotegravir and Rilpivirine (**CABENUVA**) was purchased from a local pharmacy store. Rankem in India provided all of the chemicals and buffers utilised in this method like Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen Ortho phosphate buffer, Ortho-phosphoric acid, Distilled water.

Instruments:

For the development and validation method, an automated sample injector was employed with a WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of buffer:

0.1% OPA Buffer: 1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of Standard stock solutions: Accurately Weighed and transferred 150mg of Rilpivirine, and 100mg of Cabotegravir working Standards into a 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (3000µg/ml Rilpivirine, and 2000µg/ml of Cabotegravir)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (300µg/ml Rilpivirine of and 200µg/ml of Cabotegravir)

Preparation of Sample stock solutions: Pippete 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, and 2000 µg/ml of Cabotegravir).

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (300µg/ml of Rilpivirine and 200µg/ml of Cabotegravir)

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation of Remogliflozin and Teneligliptin drug material in accordance with the ICH criteria.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: stock solutions of Cabotegravir and Rilpivirine is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Preparation of Standard stock solutions: Accurately Weighed and transferred 150mg of Rilpivirine, and 100mg of Cabotegravir working Standards into a 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (3000µg/ml Rilpivirine, and 2000µg/ml of Cabotegravir)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Rilpivirine, Cabotegravir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Rilpivirine, Cabotegravir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Rilpivirine (300ppm) and Cabotegravir (200ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Degradation studies:

Oxidation: To 1 ml of stock solution of Rilpivirine and Cabotegravir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was

diluted to obtain 300µg/ml & 200µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Rilpivirine and Cabotegravir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 300µg/ml & 200µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Rilpivirine and Cabotegravir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 300µg/ml & 200µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 300µg/ml & 200µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 3000µg/ml & 2000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 300µg/ml 200µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr at a temperature of 60º. For HPLC study, the resultant solution was diluted to 300µg/ml & 200µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS:

Table 1. System suitability table

S.No.	Rilpivirine			Cabotegravir			
Inj	**RT (min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resolution**
1	2.904	6600	1.23	3.452	9301	1.31	3.80
2	2.905	6645	1.24	3.452	9391	1.31	3.8
3	2.905	6621	1.24	3.453	9496	1.33	3.7
4	2.908	6773	1.25	3.456	9901	1.33	3.8
5	2.928	6791	1.23	3.482	9485	1.29	3.8
6	2.950	6562	1.24	3.518	9437	1.31	3.9

Table 2. Specificity data

Sample name	Retention time (Mins)	Area
Cabotegravir	2.950	2926344
Rilpivirine	3.518	3560463

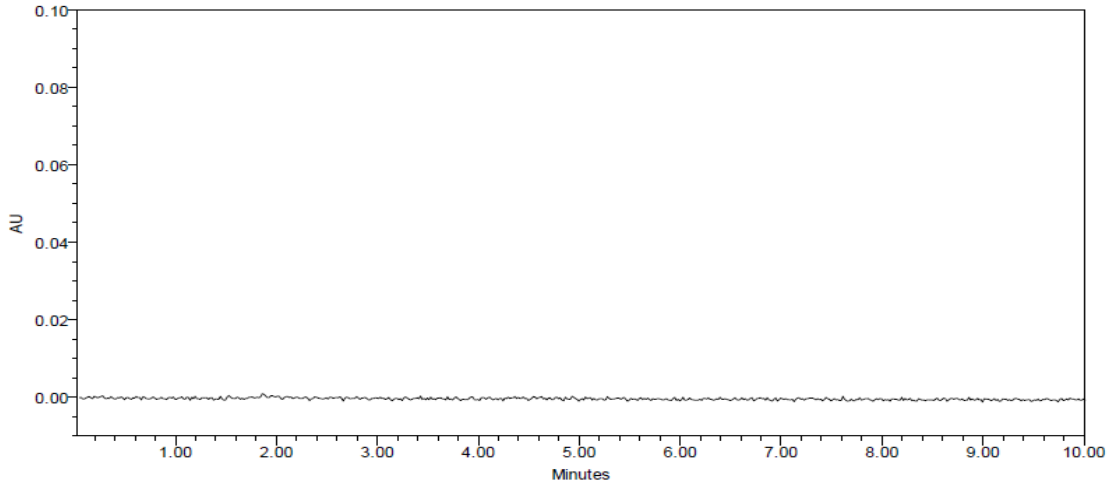

Figure 3. Blank Chromatogram

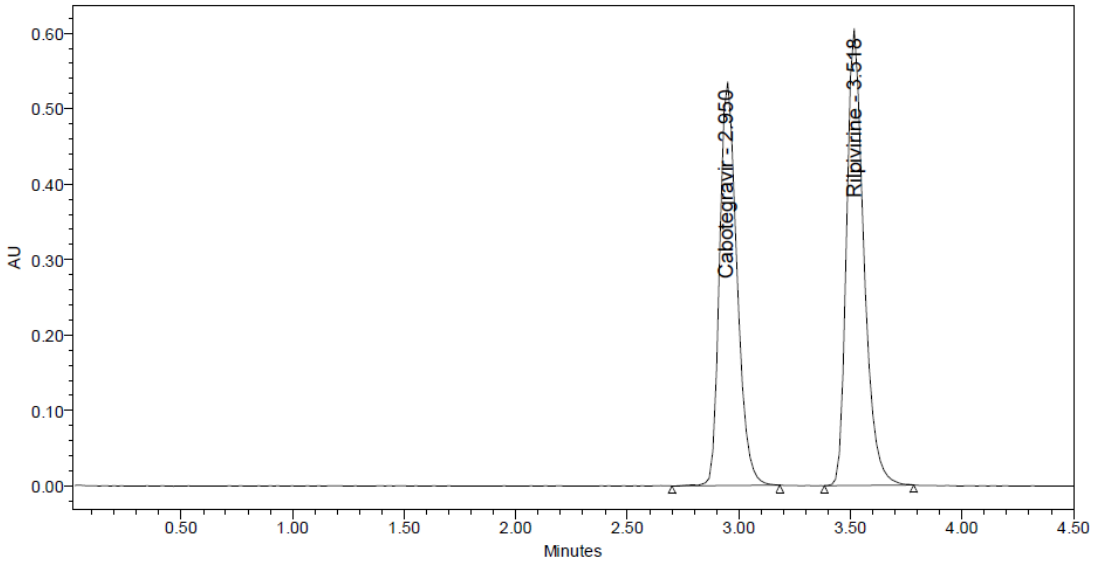

Figure 4. Specificity Chromatograms of Cabotegravir and Rilpivirine

Linearity

Table 3. Linearity table for Bilastine and Montelukast:

Cabotegravir		Rilpivirine	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	**0**	0
50	612692	**75**	855164
100	1292004	**150**	1655467
150	1983700	**225**	2540136
200	2648572	**300**	3332570
250	3207198	**375**	4139110
300	3840069	**450**	5017599

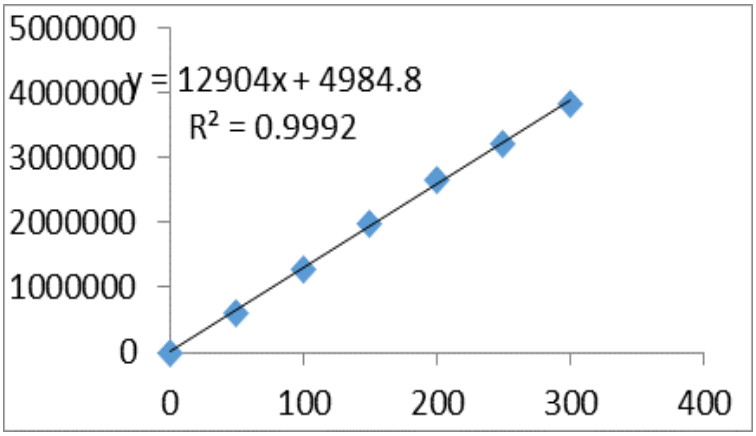

Figure 6. Calibration curve of Cabotegravir

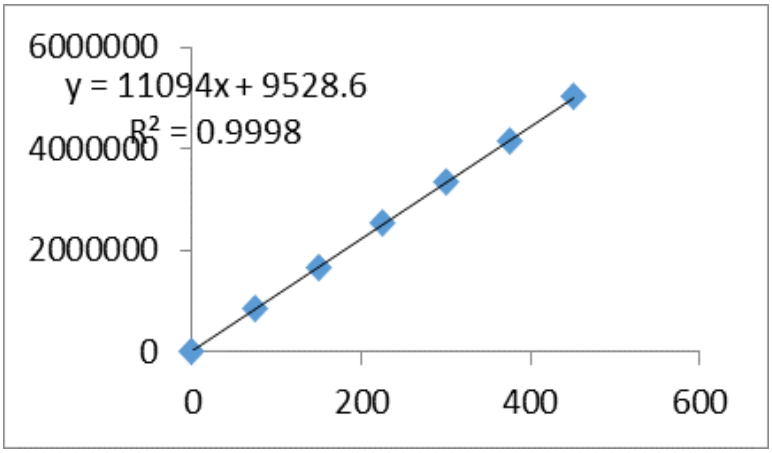

Figure 7. Calibration curve of Rilpivirine

Accuracy:

Table 4: Accuracy table of Cabotegravir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	50.55	99.88	**99.63%**
	50	50.04	100.08	
	50	49.53	99.06	
100%	100	99.25	99.25	
	100	99.83	99.83	
	100	99.07	99.07	
150%	150	150.37	100.25	
	150	148.89	99.26	
	150	150.02	100.01	

Table 5. Accuracy table of Rilpivirine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	150	148.12	98.74	**98.90%**
	150	148.45	98.97	
	150	149.99	99.99	
100%	300	297.86	99.29	
	300	298.20	99.40	
	300	293.57	97.86	
150%	450	443.94	98.65	
	450	442.33	98.30	
	450	445.03	98.90	

System Precision: With regard to the working strength of Cabotegravir and Rilpivirine, six duplicate injections of the standard solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 6: System precision

S. No	Area of Cabotegravir	Area of Rilpivirine
1.	2631326	3251030
2.	2632272	3189535
3.	2641564	3229276
4.	2660079	3264240
5.	2633799	3179537
6.	2592280	3272005
Mean	**2631887**	**3230937**
S.D	**22186.8**	**38879.9**
%RSD	**0.8**	**1.2**

The % RSD for the peak areas of Cabotegravir and Rilpivirine obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Cabotegravir and Rilpivirine allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Table 7. Method precision

S. No	Area of Cabotegravir	Area of Rilpivirine
1.	2609346	3253887
2.	2598605	3248542
3.	2633491	3197201
4.	2601683	3258275
5.	2614810	3196741
6.	2612297	3245148
Mean	**2611705**	**3233299**
S.D	**12347.0**	**28495.5**
%RSD	**0.5**	**0.9**

Results shows, the % RSD of Repeatability study was within the range for Cabotegravir and Rilpivirine is (<2%)

Table 8. Robustness

S.No.	Condition	%RSD of Cabotegravir	%RSD of Rilpivirine
1	**Flow rate (-) 0.9ml/min**	0.6	0.3
2	**Flow rate (+) 1.1ml/min**	0.8	0.9
3	**Mobile phase (-) 60B:40A**	1.1	0.5
4	**Mobile phase (+) 70B:30A**	0.3	0.5
5	**Temperature (-) 25°C**	0.5	0.3
6	**Temperature (+) 35°C**	0.6	1.0

Table 9. Forced degradation for Cabotegravir and Rilpivirine

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

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table 10. Degradation results of Cabotegravir and Rilpivirine

Type of degradation	Cabotegravir		Rilpivirine	
	%Recovered	% Degraded	%Recovered	% Degraded
Acid	93.97	6.03	94.42	5.58
Base	95.67	4.33	95.07	4.93
Peroxide	96.90	3.10	95.89	4.11
Thermal	97.68	2.32	97.20	2.80
Uv	98.89	1.11	98.63	1.37
Water	99.44	0.56	98.98	1.02

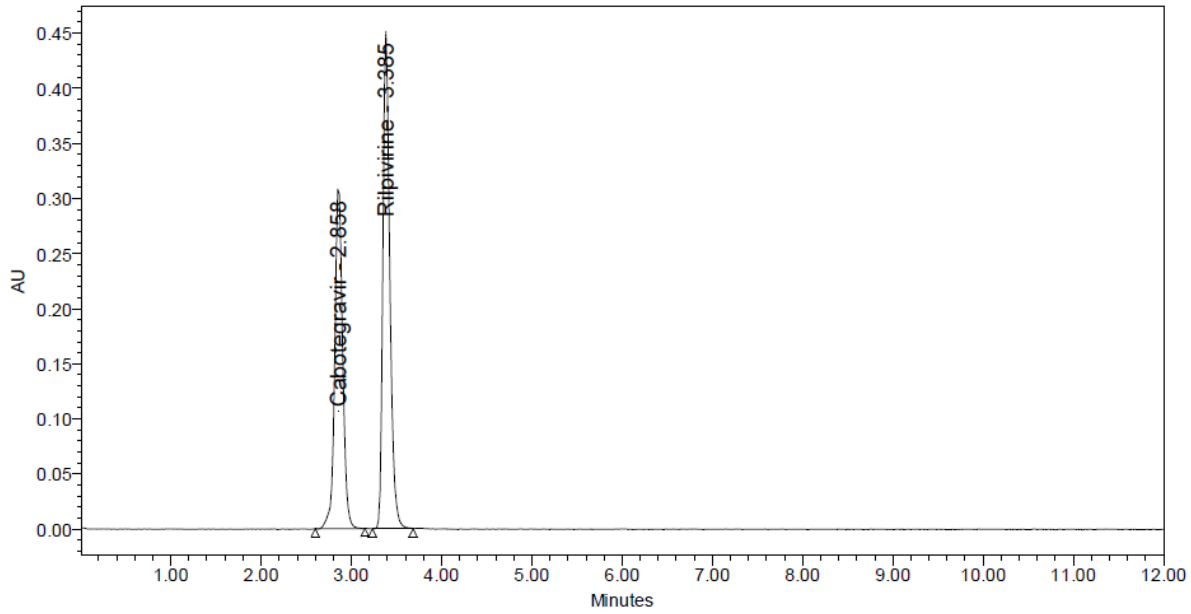

Figure 8. Acid chromatogram of Cabotegravir and Rilpivirine

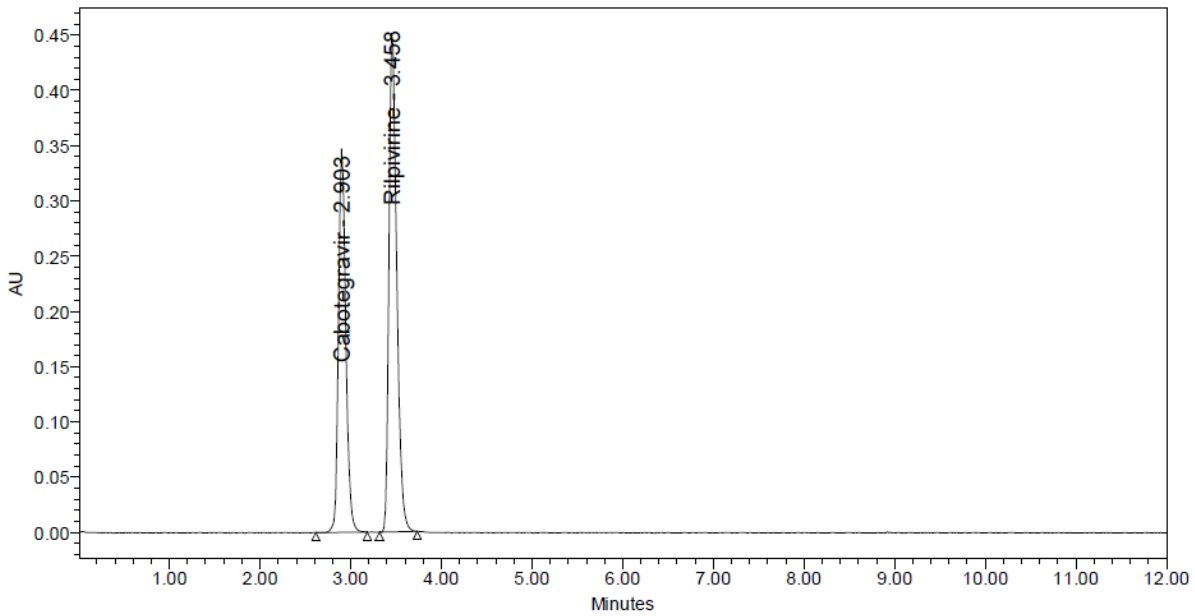

Figure 9. Base chromatogram of Cabotegravir and Rilpivirine

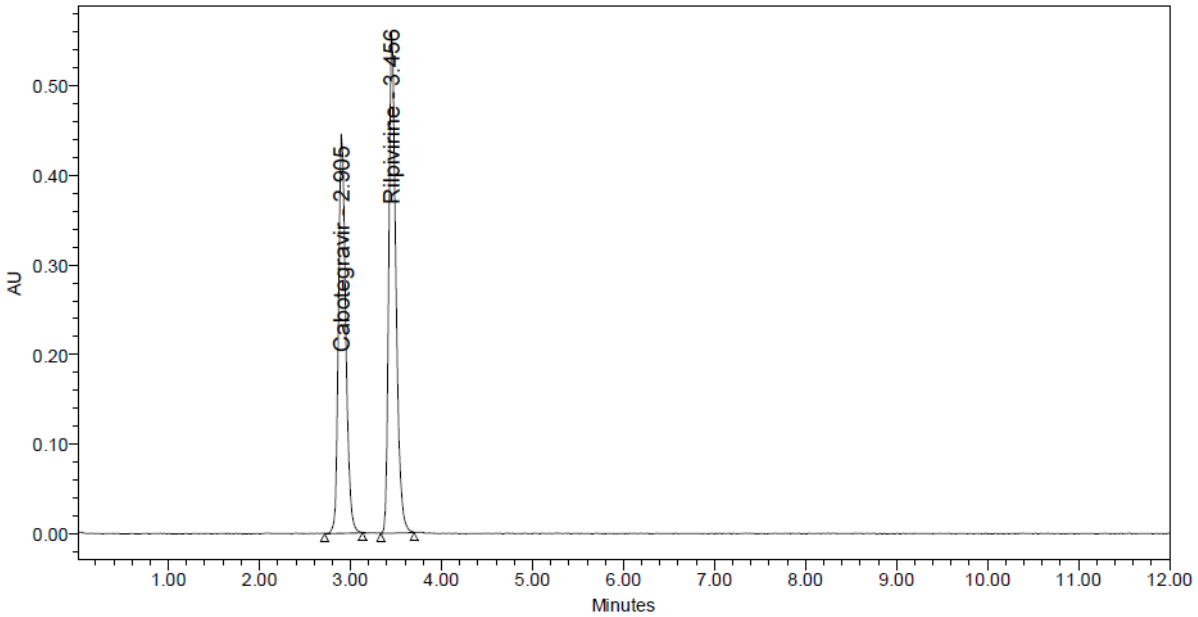

Figure 10. Peroxide chromatogram of Cabotegravir and Rilpivirine

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: (CABENUVA) bearing label claim, **Cabotegravir 400mg and Rilpivirine 600mg**, assay was carried out by injecting sample into HPLC System.

Table 11. Assay Data of Cabotegravir:

S.no	Standard Area	Sample area	% Assay
1	2631326	2609346	98.95
2	2632272	2598605	98.54
3	2641564	2633491	99.86
4	2660079	2601683	98.65
5	2633799	2614810	99.15
6	2592280	2612297	99.06
Avg	**2631887**	**2611705**	**99.03**
Stdev	**22186.8**	**12347.0**	**0.47**
%RSD	**0.8**	**0.5**	**0.5**

Table 12. Assay Data of Rilpivirine

S.no	Standard Area	Sample area	% Assay
1	3251030	3253887	100.51
2	3189535	3248542	100.34
3	3229276	3197201	98.76
4	3264240	3258275	100.64
5	3179537	3196741	98.74
6	3272005	3245148	100.24
Avg	**3230937**	**3233299**	**99.87**
Stdev	**38879.9**	**28495.5**	**0.88**
%RSD	**1.2**	**0.9**	**0.88**

Table 13. Assay outcome for Cabotegravir and Rilpivirine

Drug Name	Label claim dose	%Assay	Brand Name
Cabotegravir	400mg	99.03	**CABENUVA**
Rilpivirine	600mg	99.87	

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Cabotegravir and Rilpivirine in tablet dosage form. The method was found to be accurate, precise, robust and specific. Retention time of Cabotegravir and Rilpivirine were found to be 2.950 min and 3.518. %RSD of the Cabotegravir and Rilpivirine were and found to be 0.8 and 0.5 respectively. %Recovery was obtained as 99.19% and 98.90% for Cabotegravir and Rilpivirine respectively. LOD, LOQ values obtained from regression equations of Cabotegravir and Rilpivirine were 0.91, 2.77 and 1.05, 3.17 respectively. Regression equation of Cabotegravir is y = 12904x + 4984.8, and y = 11094x + 9528.6 of Rilpivirine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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