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RP - HPLC Analytical Method Development and Validation for the Simultaneous Estimation of Cabotegravir and Rilpivirine In Bulk and Tablet Dosage Form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Rilpivirine and Cabotegravir in dosage form. Chromatogram was run through AgilentC18 150 x 4.6mm, 5.0µm. [(1)] Mobile phase containing Buffer 0.1% Ammonium Acetate: Acetonitrile taken in the ratio 55:45v/v was pumped through the column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. The optimized wavelength selected was 260 nm. The retention time of Rilpivirine and Cabotegravir was found to be 2.238 min and 2.953 min. %RSD of the Rilpivirine and Cabotegravir was found to be 0.5 and 0.5 respectively. %Recovery was obtained as 99.85% and 99.84% for Rilpivirine and Cabotegravir respectively. LOD and LOQ values obtained from regression equations of Rilpivirine and Cabotegravir were 0.11, 0.3,2, and 0.02, 0.06 respectively. [(2)] The regression equation of Rilpivirine is y = 17712x + 2324.3 and y = 17293x + 1410.5 of Cabotegravir 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.

Keywords: Rilpivirine, Cabotegravir, RP-HPLC, Validation.

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INTRODUCTION

Liquid chromatography is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the time of the solutes through a column. [(3)] During this time pressure liquid chromatography began to be used to decrease flow through time, thus reducing the separation time of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have a constant flow rate or constant pressure was debated. High-pressure liquid chromatography quickly improved with the development of column packing materials. Additional convenience of online detectors became rapidly a powerful separation technique and is today called High-Performance Liquid Chromatography (HPLC). [(5)]

Cabotegravir:

Cabotegravir is an HIV-1 integrase inhibitor that is prescribed with the non-nucleoside reverse transcriptase inhibitor, Rilpivirine.4,5,6 Early research into Cabotegravir showed it had lower oral bioavailability than dolutegravir. The development of Cabotegravir was later developed to create a long-acting monthly intramuscular injection. Cabotegravir is an inhibitor of HIV integrase, which reduces viral replication. It has a long duration of action as the oral is given daily and the intramuscular suspension is given monthly. Patients should be counseled regarding the risk of hypersensitivity, hepatotoxicity, and depression. Cabotegravir binds to the active site of HIV integrase, preventing strand transfer of the viral genome into the host genome, and preventing replication of the virus. [(6)]

Rilpivirine:

Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is used for the treatment of HIV-1 infections in treatment-naive patients. It is a diaryl pyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance of Rilpivirine is less likely to develop than other NNRTIs. FDA approved on May 20, 2011. Rilpivirine is the most potent NNRTI and has an EC50 of 0.73 nM in vitro against HIV-1 because its chemical structure allows for better binding to reverse transcriptase. Rilpivirine is an NNRTI that binds to reverse transcriptase which results in a block in RNA and DNA-dependent DNA polymerase activities. One such activity is HIV-1 replication. Intracellular phosphorylation is not necessary for its antiviral activity. [(5)] Because the structure of Rilpivirine is

flexible around the aromatic rings, the molecule can have multiple conformations so that can bind to residues in the reverse transcriptase enzyme which has a lower mutation rate. [4]

MATERIALS AND METHOD

Materials and reagents:

Rilpivirine and Cabotegravir pure drugs (API), Combination Rilpivirine and Cabotegravir formulation (Cabenuva), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem. [7]

Instrumentation:

S.no	Instrument	Model
1	HPLC	WATERS HPLC,2695 System, software : Empower 2 software, PDA detector
2	UV/ VIS spectrophotometer	UV Win 6 software
3	pH meter	BVK Enterprises, India
4	Electronic balance	Denver
5	Ultrasonicator	BVK enterprises
6	Beakers	Borosil

PREPARATION OF MOBILE PHASE:

Preparation of Standard stock solutions:

Accurately weighed 60 mg of Rilpivirine, 40 mg of Cabotegravir and transferred to 100 ml volumetric flask. 3/4th of the diluents were added to the flask and sonicated for 10 minutes. The flask was made up of diluent and labeled as Standard stock solution_1. (600µg/ml of Rilpivirine and 400µg/ml of Cabotegravir).

Preparation of Standard working solutions (100% solution):

From the above solution 1ml 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 stock solutions:

1ml vial of injection was taken that is equivalent to 300MG, 200MG/1ml of dosage form was transferred into a 250 ml volumetric flask, 100ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1200µg/ml of Rilpivirine and 800µg/ml of Cabotegravir).

Preparation of Sample working solutions (100% solution):

0.5ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with diluent. (60µg/ml Rilpivirine of and 40µg/ml of Cabotegravir).

VALIDATION OF METHOD DEVELOPED:

System Suitability:

The system suitability parameters were determined by preparing standard solutions of Rilpivirine 60 µg/ml and Cabotegravir 40 µg/ml. The solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. [8] The % RSD for the area of six standard injections results should not be more than 2%.

Specificity:

Specificity of a method was determined by testing standard substances against potential interferences. There should not be interfering peaks in the blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity:

By appropriate aliquots of the standard Rilpivirine and Cabotegravir prepared six working solutions ranging between 15-90µg/mL& 10-60µg/ml. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficient on curves for Rilpivirine and Cabotegravir. [9]

Precision:

The repeatability of the method was verified by calculating the % RSD of six replicate injections of 100% concentration (60µg/ml of Rilpivirine and 40µg/ml of Cabotegravir) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days.

Accuracy:

Accuracy was carried out by % recovery studies of Rilpivirine and Cabotegravir at three different concentration levels (50%, 100%, and 150%). Percentage recovery was calculated from the amount added and the amount recovered. The percentage recovery was within the acceptance criteria, this indicates the accuracy of the method.

Acceptance criteria:

% recovery for each level should be between 98 to 102.

Robustness:

Robustness of the method was verified by altering the chromatographic conditions like flow rate, mobile phase ratio and temperature, but there was no recognized change in the result and all are within range as per ICH guidelines. Robustness conditions like flow minus (0.9 ml/min), flow plus (1.1 ml/min), 50:50mobile phase minus 60:40 mobile phase plus, temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in a duplicate manner. System

suitability parameter was passed. % RSD was within the limit. [11]

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s.

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 0.3ml of each of the Standard stock solution, solutions was transferred to 10ml volumetric flasks and made up with the same diluents. [10]

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 0.9ml of each of the Standard stock solution respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

DEGRADATION STUDIES:

Acid degradation:

To 1 ml of stock solution of Rilpivirine and Cabotegravir, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60 °C. The resultant solution was diluted to obtain 60µg/ml and 40µg/ml solutions and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Oxidative Degradation:

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 60°C. For the HPLC study, the resultant solution was diluted to obtain 60µg/ml and 40µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. [9]

Alkali Degradation:

To 1 ml of stock solution of Rilpivirine and Cabotegravir, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 60µg/ml and 40µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Thermal Degradation:

The standard drug solution was placed in an oven at 105°C for 6Hrs to study dry heat degradation. For the HPLC study, the resultant solution was diluted to 60µg/ml and 40µg/ml solution and 10µl

were injected into the system and the chromatograms were recorded to assess the stability of the sample. [12]

Photo Degradation:

The photochemical stability of the drug was also studied by exposing the 600µg/ml and 400µg/ml solution to UV light by keeping the beaker in a UV chamber for 7 days or 200 Watt hrs/m2 in a photo stability chamber. For the HPLC study, the resultant solution was diluted to obtain 60µg/ml and 40µg/ml solutions and 10µl was injected into the system and the chromatograms were recorded to assess the stability of the sample. [13]

RESULTS AND DISCUSSION:

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table: 1 System suitability parameters for Rilpivirine and Cabotegravir

S no	Rilpivirine			Cabotegravir			
Inj	Rt(min)	USP Plate Count	Tailing	Rt(min)	USP Plate Count	Tailing	Resolution
1	2.231	2891	1.32	2.947	4160	1.26	4.0
2	2.232	2899	1.29	2.948	4052	1.29	3.9
3	2.232	2954	1.29	2.948	4113	1.29	3.9
4	2.234	2891	1.32	2.948	4073	1.26	4.0
5	2.236	2966	1.33	2.951	3995	1.28	3.9
6	2.244	2905	1.29	2.962	4116	1.25	4.0

Retention times of Rilpivirine and Cabotegravir were 2.238 min and 2.953 min respectively. We did 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.

Specificity:

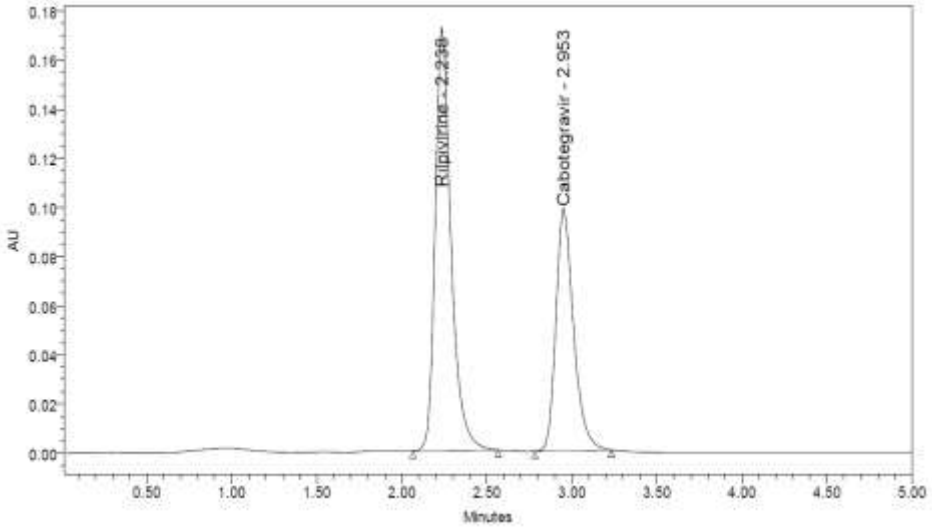

Figure 1: Optimized chromatogram

Linearity:

Table 2: Linearity table for Rilpivirine and Cabotegravir.

Rilpivirine		Cabotegravir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
15	264514	10	176169
30	535681	20	344239
45	795817	30	523644
60	1082007	40	695784
75	1331010	50	861876
90	1586616	60	1039696

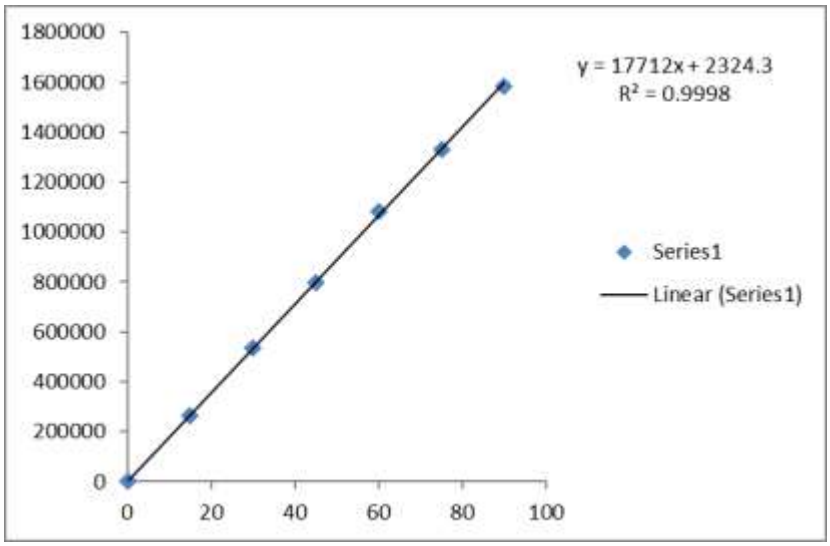

Figure 2: Calibration curve of Rilpivirine

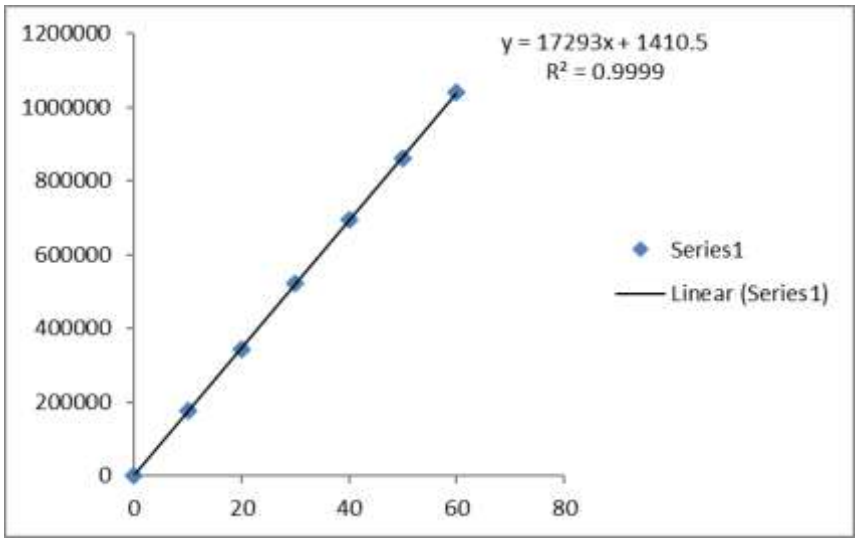

Figure 3: Calibration curve of Cabotegravir

Six linear concentrations of Rilpivirine (15-90µg/ml) and Cabotegravir (10- 60µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations

obtained for Rilpivirine was y = 17293x + 1410.5 and for Cabotegravir was y = 17293x + 1410.5. The correlation coefficient obtained was 0.999 for the two drugs.

Precision:

System Precision:

Table 3: System precision table of Rilpivirine and Cabotegravir

S. No	Area of Rilpivirine	Area of Cabotegravir
1.	1084716	691648
2.	1073164	701434
3.	1070111	697128
4.	1074120	692819
5.	1076898	693726
6.	1073858	693384
Mean	1075478	695023
S.D	5019.7	3637.3
%RSD	0.5	0.5

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation, and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.5% respectively for Rilpivirine and Cabotegravir. As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability:

Table 4: Repeatability table of Rilpivirine and Cabotegravir

S. No	Area of Rilpivirine	Area of Cabotegravir
1.	1083980	694493
2.	1071806	690160
3.	1068637	691865
4.	1072581	690852
5.	1073888	698252
6.	1068931	699756
Mean	1073304	694230
S.D	5621.2	4008.8
%RSD	0.5	0.6

Multiple sampling from a sample stock solution was done and six working sample solutions of the 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.

Accuracy:

Table 5: Accuracy table of Rilpivirine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean
50%	30	29.9	99.7	99.86%
	30	29.7	99.1	
	30	30.2	100.8	
100%	60	60.4	100.7	100.1%
	60	59.6	99.3	
	60	60.2	100.3	
150%	90	90.1	100.1	99.95%
	90	89.5	99.5	
	90	90.1	100.1	

Table 6: Accuracy table of Cabotegravir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean
50%	20	19.93	99.66	99.80%
	20	20.08	100.41	
	20	19.87	99.34	
100%	40	40.10	100.26	100.09%
	40	39.69	99.22	
	40	40.03	100.08	
150%	60	59.82	99.70	99.84%
	60	59.98	99.96	
	60	59.93	99.89	

Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.95% and 99.84% for Rilpivirine and Cabotegravir respectively.

Sensitivity:

Table 7: Sensitivity table of Rilpivirine and Cabotegravir

Molecule	LOD	LOQ
Rilpivirine	0.11	0.32
Cabotegravir	0.02	0.06

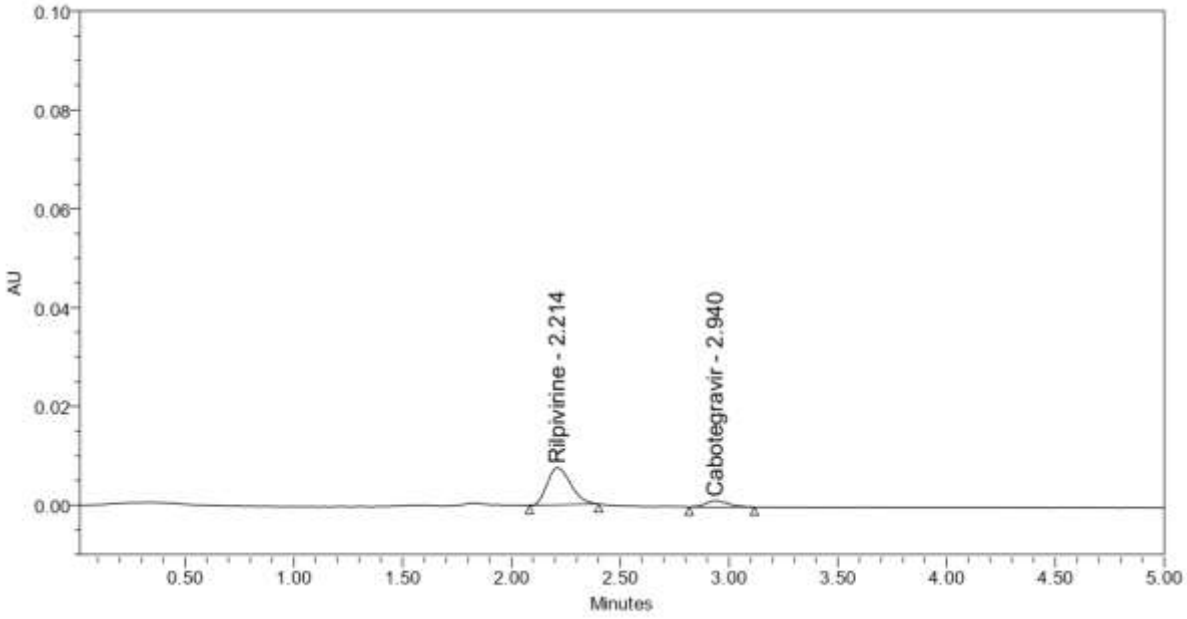

Figure 4 : LOD Chromatogram of Standard

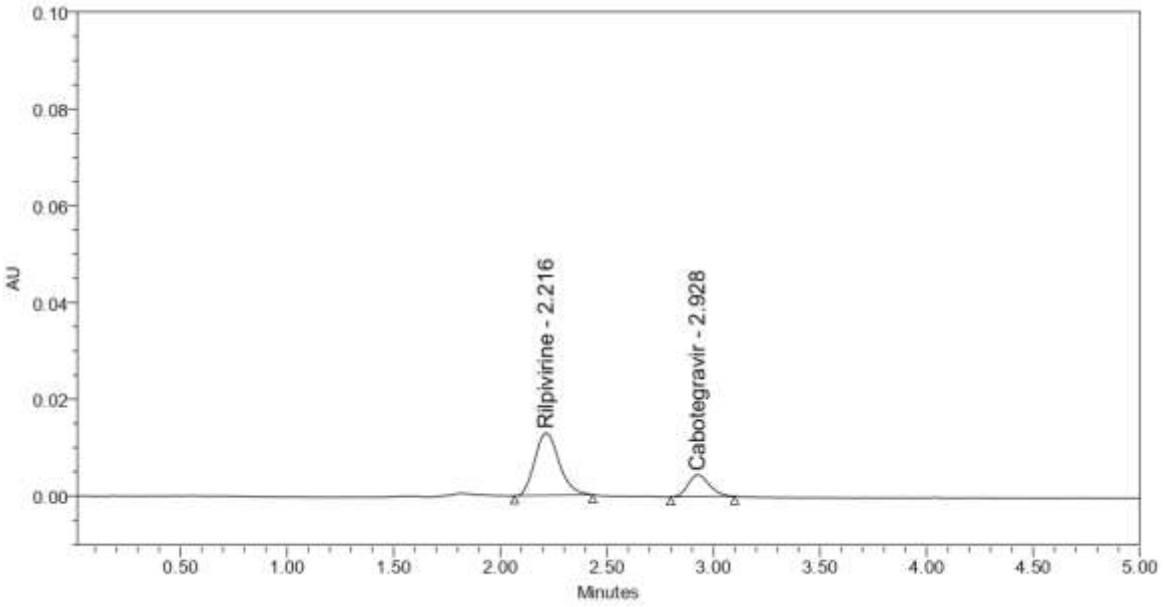

Figure 5: LOQ Chromatogram of Standard

Robustness:

Table 8: Robustness data for Rilpivirine and Cabotegravir.

S.no	Condition	%RSD of Rilpivirine	%RSD of Cabotegravir
1	Flow rate (-) 0.9ml/min	0.4	0.5
2	Flow rate (+) 1.1ml/min	0.1	0.2
3	Mobile phase (-) 50B:50A	0.5	0.4
4	Mobile phase (+) 60B:40A	0.2	0.3
5	Temperature (-) 25°C	0.9	0.3
6	Temperature (+) 35°C	0.2	0.3

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (50B:50A), mobile phase plus (60B:40A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay:

Cabenuva, bearing the label claim Cabotegravir and Rilpivirine 300MG, 600MG/1ml. Assay were performed with the above formulation. The average % Assay for Rilpivirine and Cabotegravir obtained was 99.70% and 99.79% respectively.

Table 9: Assay Data of Rilpivirine

S.no	Standard Area	Sample area	% Assay
1	1084716	1083980	100.69
2	1073164	1071806	99.56
3	1070111	1068637	99.26
4	1074120	1072581	99.63
5	1076898	1073888	99.75
6	1073858	1068931	99.29
Avg	1075478	1073304	99.70
Stdev	5019.7	5621.2	0.52
%RSD	0.5	0.5	0.5

Table 10: Assay Data of Cabotegravir

S.no	Standard Area	Sample area	% Assay
1	691648	694493	99.82
2	701434	690160	99.20
3	697128	691865	99.45
4	692819	690852	99.30
5	693726	698252	100.36
6	693384	699756	100.58
Avg	695023	694230	99.79
Stdev	3637.3	4008.8	0.58
%RSD	0.5	0.6	0.58

DEGRADATION DATA :

Table 11: Degradation data

Type of degradation	Rilpivirine			Cabotegravir		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	1041684	96.76	3.24	668622	96.11	3.89
Base	990182	91.98	8.02	654187	94.03	5.97
Peroxide	1021463	94.88	5.12	667881	96.00	4.00
Thermal	1061407	98.59	1.41	680022	97.74	2.26
UV	1075369	99.89	0.11	684046	98.32	1.68
Water	1070311	99.42	0.58	690915	99.31	0.69

CONCLUSION:

A simple, Precise, and Accurate method has been developed for the simultaneous quantification of Cabotegravir and Rilpivirine in a pharmaceutical dosage form. Retention time of Rilpivirine and Cabotegravir were found to be 2.238 min and 2.953 min. Regression equation of Rilpivirine is y = 17712x + 2324.3 and y = 17293x + 1410.5 of Cabotegravir. %RSD of the Rilpivirine and Cabotegravir were found to be 0.5 and 0.5 respectively. %Recovery was obtained as 99.85% and 99.84% for Rilpivirine and Cabotegravir respectively. LOD and LOQ values obtained from regression equations of Rilpivirine and Cabotegravir were 0.11,0.32 and 0.02, 0.06 respectively. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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