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Research Article

A Validated Stability Indicating RP-HPLC Method Development for Simultaneous estimation of Cabotegravir and Rilpivirine in Pharmaceutical Dosage form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cabotegravir and Rilpivirine in pharmaceutical dosage form. Chromatogram was run through Kromasil C18 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.01N Potassium dihydrogen phosphate: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.01N Kh2PO4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 257 nm. Retention time of Rilpivirine and Cabotegravir were found to be 2.257 min and 2.642min. %RSD of the Rilpivirine and Cabotegravir were and found to be 0.5 and 1.4 respectively. %Recovery was obtained as 100.43% and 100.13% for Rilpivirine and Cabotegravir respectively. LOD, LOQ values obtained from regression equations of Rilpivirine and Cabotegravir were 0.18, 0.54 and 0.15, 0.46 respectively. Regression equation of Cabotegravir is y = 9571.x + 4414, and y = 5378.x + 919.7 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.

Keywords: Cabotegravir, Rilpivirine, RP-HPLC

INTRODUCTION

Cabotegravir was approved on January 2021, it is an antiretroviral drug, a structural analogue of dolutegravi**r**. 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. It has a long duration of action as the oral tablet is given daily and the intramuscular suspension is given monthly. Rilpivirine. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA pyrimidine, Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients. Because of its flexible chemical structure, resistance of Rilpivirine is less likely to develop than other NNRTI's.

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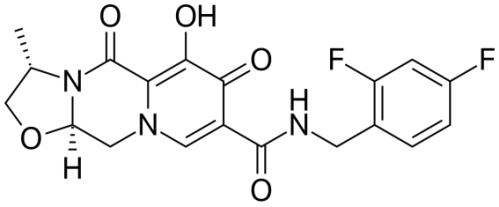

Figure 1: Structure of Cabotegravir

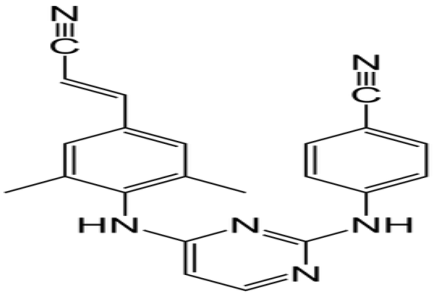

Figure 2: Structure of Rilpivirine

Literature survey revealed that there are some methods reported for simultaneous estimation of individual drugs or some methods for estimation of individual drugs or with other drugs. UV-spectrophotometry methods RP-HPLC on the basis of the stability indicting simultaneous estimation of Cabotegravir and Rilpivirine by RP-HPLC in pharmaceutical dosage form. The main of this study is to develop a simple, accurate relatively sensitive and rapid RP- HPLC technique for estimation of cabotegravir and Rilpivirine in bulk and pharmaceutical dosage. A validated method also applied for cabotegravir and Rilpivirine estimation asper the ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents: spectrum pharma has provided Cabotegravir and Rilpivirine pure drugs. The combination injection Cabotegravir and Rilpivirine (Cabenuva) was purchased from local pharmacy Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments and Chromatographic Conditions: Electronics Balance-Denver, PH meter-BVK enterprises, India, Ultrasonicator-BVK enterprises, Waters HPLC 2695 System equipped with quaternary pumps, Photo Diode Array detector and Auto sampler, UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of cabotegravir and Rilpivirine solutions. kromasil C18 (4.6×150 mm, 5µm) was used for separation. The data was acquired at 257 nm. The output signal was acquired by using Empower 2 software.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in he ratio of 50:50(v/v).

Preparation of Standard stock solutions:
Accurately weighed and transferred 37.5mg of Rilpivirine, and 25mg of Cabotegravir working Standards into a 50ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (750µg/ml Rilpivirine, and 500µg/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. (75µg/ml Rilpivirine of and 50µg/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. (750µg/ml Rilpivirine of and 500µg/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. (75µg/ml Rilpivirine of and 50µg/ml of Cabotegravir)

Preparation of buffer:

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

Buffer: (0.01N Kh2po4)
Accurately weighed 1.42gm 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 th water then PH adjusted to 4.0 with dil. Orthophosphoric acid solution.

Method Validation
As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

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.

Accuracy:

Preparation of Standard stock solutions: Accurately Weighed and transferred 37.5mg of Rilpivirine, and 25mg of Cabotegravir working Standards into a 50ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (750µg/ml Rilpivirine, and 500µg/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.

Robustness:

Robustness conditions like Flow minus (1ml/min), Flow plus (1lus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected 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.

Degradation studies:

Oxidation: To 1 ml of stock solution of Rilpivirine and Cabotegravir, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 75µg/ml & 50µg/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 75µg/ml & 50µg/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, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 75µg/ml & 50µg/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 75 µg/ml & 50 µg/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 1500µg/ml & 1000µg/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 75µg/ml & 50µg/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 1h r s at a temperature of 60º. For HPLC study, the resultant solution was diluted to 75µg/ml & 50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Cabotegravir (50ppm) and Rilpivirine(75ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Assay of Cabenuva: assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system.

RESULTS & DISCUSSION

Optimization of chromatographic conditions: To develop and establish a suitable RP- HPLC Method for estimation of cabotegravir and Rilpivirine in bulk and pharmaceutical dosage form, Different preliminary tests were performed and different chromatographic conditions were developed which were given in table -1. The final analysis was performed by using 0.01N potassium dihydrogen phosphate and acetonitrile (60:40) v/v at a flow rate of 1ml/min, sample were analysed at 257 nm using kromasil (150×4.6mm,5µm) with run time of 10 min. The proposed method was optimized to give Sharpe peak and resolution and the optimised chromatogram was obtained as shown in (figure)

Validation: Linearity was established at Six linear concentration of Cabotegravir (18.75-112.5µg/ml) and Rilpivirine (12.5- 75µg/ml) were injected in a duplicate manner. Average areas were mentioned and linearity equations obtained for cabotegravir was y = 9571x + 4414.2. and of cabotegravir was y = 5378.6x + 848.3 Correlation coefficient obtained was 0.999 for the both drugs. The linearity calibration curves were plotted as shown in (figure-4,5) Retention time of cabotegravir was 2.642min and Rilpivirine was 2. 257min.No interfering peaks in blank and placebo were found in this method. So, this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared by standard addition method. Triplicates injections were given % Recovery was obtained as 100.43% for cabotegravir and 100.13% for Rilpivirine. and % RSD for system precision for Cabotegravir was 1.4 % and for Rilpivirine was 0.5%. %RSD for repeatability for Cabotegravir was 0.9% and for Rilpivirine was 0.4%. %RSD for intermediate precision for cabotegravir was 1.1% and for Rilpivirine was 0.7%. Since %RSD is was less than "2" the system precision was passed in this method shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Abiraterone. The detection limit value for cabotegravir was 0.54 and for Rilpivirine was 0.18. The Quantification limit value for cabotegravir was 0.46 and for Rilpivirine was 0.15 as given in (Table-4).

Table1: Optimization Chromatographic conditions

Parameter	**Condition**
RP-HPLC	WATERSHPLC2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 software
Mobile phase	60% 0.01N Kh2po4: 40% Acetonitrile
Flow rate	1ml/min
Column	Kromasil C18 (4.6 x 150mm, 5µm)
Detector wavelength	257nm
Column temperature	30°C
Injection volume	10 µL
Run time	10min
Diluents	Water and Acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

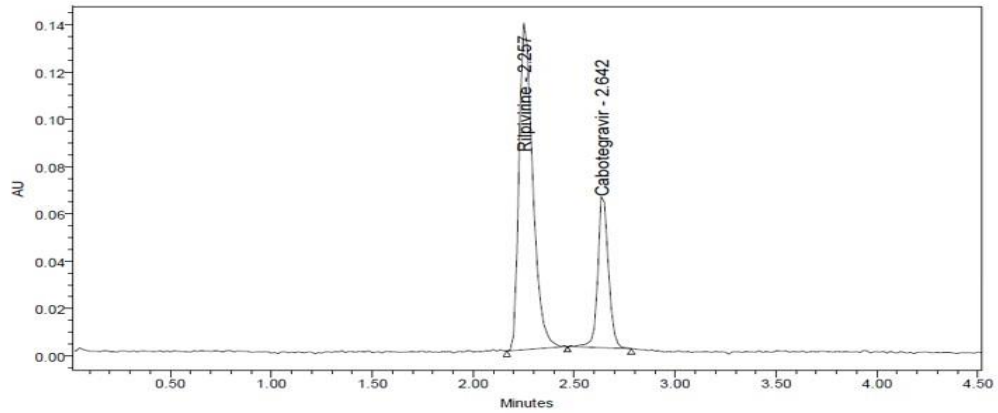

Figure3: Optimized Chromatogram

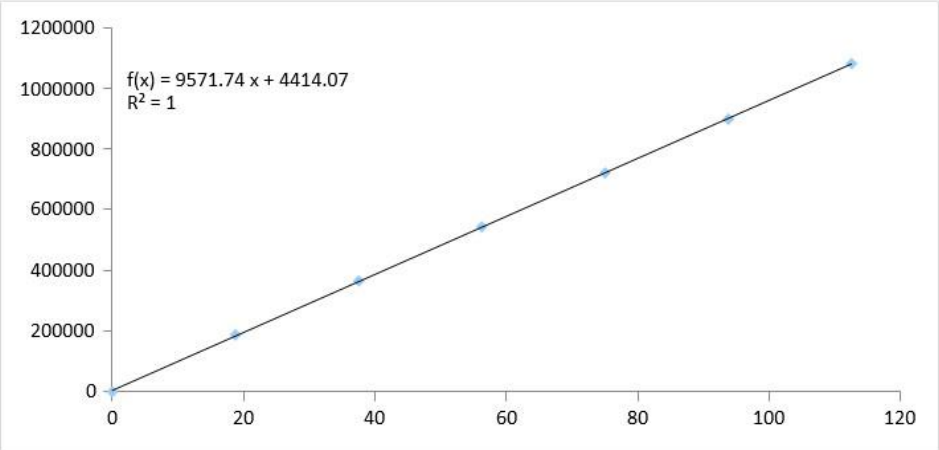

Figure-4: calibration curve of Cabotegravir

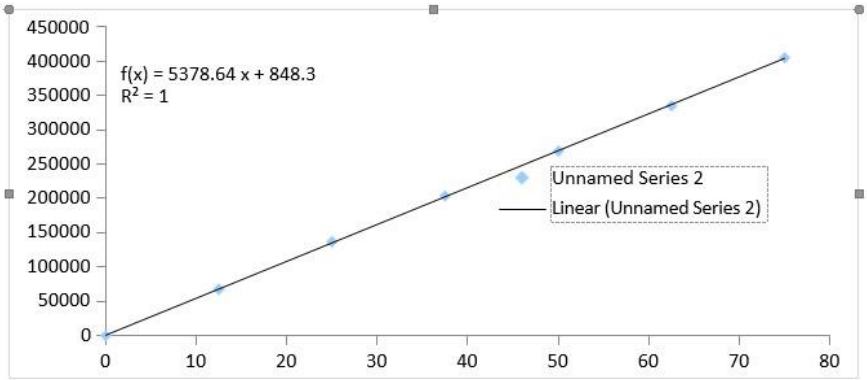

Figure-5: calibration curve of Rilpivirine

Table-2: Accuracy results of Cabotegravir (Drug1) and Rilpivirine (Drug2)

%Level	Amount Spiked (µg/ml)		Amount Recovered (µg/ ml)		% Recovery		Mean % Recovery	
	Drug 1	Drug 2	Drug 1	Drug 2	Drug 1	Drug 2	Drug 1	Drug 2
50%	37.5	25	38.3	25.04	101.8	100.17		
	37.5	25	37.6	25.18	100.4	100.73		
	37.5	25	37.8	25.00	100.9	100.02		
100%	75	50	75.6	50.33	100.8	100.66		
	75	0.5	76.1	49.81	101.5	99.61		
	75	0.5	74.9	50.03	99.8	100.06	100.43%	100.13%
150%	112.5	75	111.6	74.830	99.2	99.78		
	112.5	75	112.0	75.05	99.96	100.07		
	112.5	75	112.3	75.05	99.8	100.06		

Table-3: Precision Results of Cabotegravir and Rilpivirine

s.no	System precision		Repeatability		Intermediate precision	
	Area of Rilpivirine	Area of cabotegravir	Area of Rilpivirine	Area of cabotegravir	Area of Rilpivirine	Area of cabotegravir
1	722243	270847	722476	263484	716118	267691
2	718575	266160	724441	268267	725316	270696
3	719973	264432	718972	265650	722517	262986
4	727887	269220	725864	270404	714413	264360

5	721978	273778	722026	267438	723797	265808
6	725662	264800	719533	266792		
mean	722720	268206	722219	267006	720432	266308
S.D	3488.8	3717.0	2686.4	2350.0	4857.0	3010.3
%RSD	0.5	1.4	0.4	0.9	0.7	1.1

Table 4: Sensitivity table of Cabotegravir and Rilpivirine

Molecule	LOD	LOQ
Cabotegravir	0.18	0.15
Rilpivirine	0.54	0.46

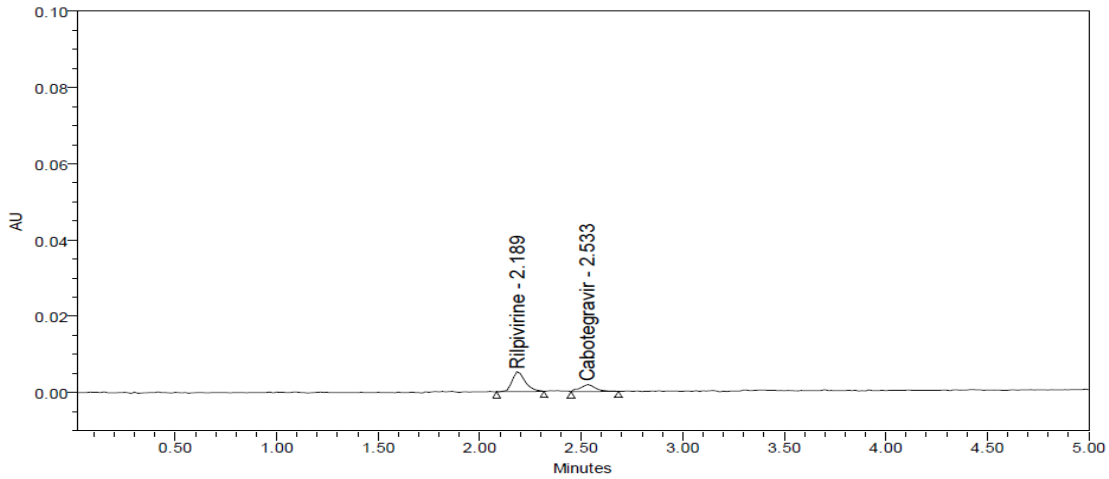

Figure 6: LOD Chromatogram of Standard

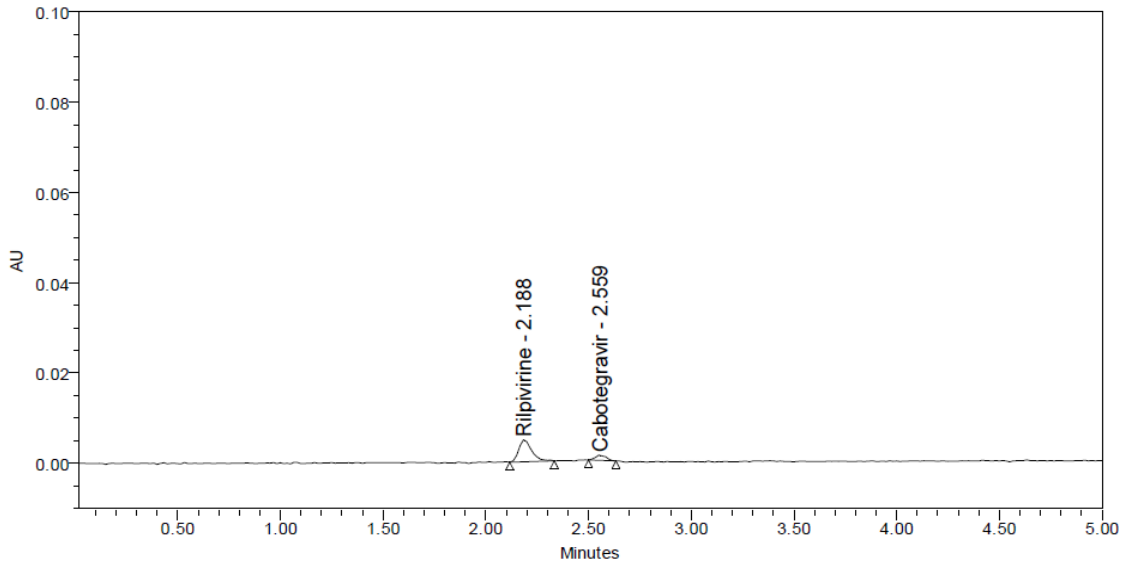

Figure 7: LOQ Chromatogram of Standard

Table 5 : Robustness data for Cabotegravir and Rilpivirine

S.no	Condition	%RSD of Cabotegravir	%RSD of Rilpivirine
1	Flow rate (-) 0.9ml/min	0.4	0.2
2	Flow rate (+) 1.1ml/min	0.4	0.5
3	Mobile phase (-) 65B:35A	0.7	0.8
4	Mobile phase (+) 55B:45A	0.1	1.0
5	Temperature (-) 27°C	0.2	0.3
6	Temperature (+) 33°C	0.4	1.1

Table-6: System Suitability Parameters for Rilpivirine and cabotegravir

S.n o	Rilpivirine			Cabotegravir			
Inje	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.209	5615	1.30	2.581	11469	1.00	3.1
2	2.225	5669	1.30	2.589	11580	1.01	3.1
3	2.248	5635	1.30	2.612	11295	1.00	3.1
4	2.250	5764	1.29	2.617	11375	1.00	3.1
5	2.251	5730	1.30	2.633	11764	1.01	3.1
6	2.263	5699	1.30	2.635	11482	1.02	3.1

Table7: Degradation data

Type of degradation	Rilpivirine			Cabotegravir		
	AREA	%RECOVE RED	% DEGRADE D	AREA	%RECOVE RED	% DEGRADE D
Acid	692911	95.78	4.22	257464	95.90	4.10
Base	685421	94.74	5.26	248852	92.69	7.31
Peroxide	693933	95.92	4.08	253609	94.46	5.54
Thermal	711938	98.41	1.59	256497	95.54	4.46
Uv	712835	98.53	1.47	259588	96.69	3.31
Water	719707	99.48	0.52	266573	99.29	0.71

Table 8: Assay Results of Rilpivirine and cabotegravir

S.no	% Assay Rilpivirine	%Assay cabotegravir
1	99.87	98.14
2	100.14	99.92
3	99.38	98.95
4	100.33	100.72
5	99.80	99.61
6	99.46	99.37
Avg	99.83	99.45
SD	0.37	0.88
%RSD	0.4	0.9

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Rilpivirine and Cabotegravir in pharmaceutical dosage form. Retention time of Rilpivirine and Cabotegravir were found to be 2.257 min and 2.642. %RSD of the Rilpivirine and Cabotegravir were and found to be 0.5 and 1.4 respectively. %Recovery was obtained as 100.43% and 100.13% for Rilpivirine and Cabotegravir respectively. LOD, LOQ values obtained from regression equations of Rilpivirine and Cabotegravir were 0.18, 0.54 and 0.15, 0.46

respectively. Regression equation of Cabotegravir is y = 9571.x + 4414, and y = 5378.x + 919.7 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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