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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CABOTEGRAVIR AND RILPIVIRINE IN TABLET DOSASE FORM

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Abstract

A simple, efficient, and time-saving reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Cabotegravir and Rilpivirine in tablet dosage form. The method is rapid, precise, sensitive, and reproducible, making it suitable for routine analysis. Chromatographic separation was performed using a Waters Alliance e2695 system equipped with a Waters XBridge Phenyl column (150 × 4.6 mm, 3.5 µm). The mobile phase consisted of ammonium formate buffer (pH 3.5, adjusted with formic acid) and acetonitrile in a 70:30 v/v ratio, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 269 nm using a photodiode array (PDA) detector at ambient temperature. The method demonstrated acceptable system suitability parameters, with the number of theoretical plates for both analytes not less than 2000 and tailing factors not exceeding 2. The relative standard deviation (RSD) of peak areas was consistently below 2.0%, indicating good repeatability. Method validation was conducted following ICH guidelines, confirming that the method is accurate, precise, robust, and cost-effective for the quantitative determination and stability analysis of Cabotegravir and Rilpivirine in pharmaceutical formulations.

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

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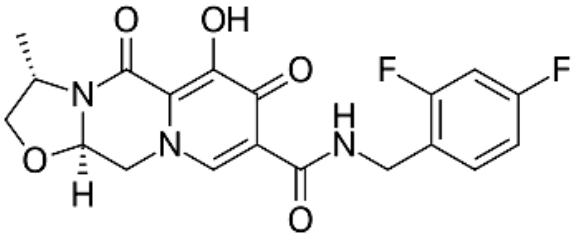
Introduction

Chromatography is a separation technique based on the differential interaction of mixture components between a stationary and a mobile phase. Analytical chromatography, particularly HPLC, is widely used to identify and quantify substances such as macromolecules, polymers, and ionic compounds. An HPLC system typically includes mobile phase reservoirs, pumps, degassers, columns, and detectors. Key performance parameters include column efficiency (N), capacity factor (K'), resolution (Rs), retention time (Rt), and peak asymmetry (As). Method development involves understanding the sample, defining separation goals, selecting detectors, performing trial runs, optimizing conditions, and validating the method. As per ICH Q2 (R1) guidelines, validation covers system suitability, specificity, linearity, accuracy, precision, robustness, LOD, and LOQ [1-3]. System Suitability: Capacity Factor- k' > 2, Injection precision-RSD < 1% for n ≥ 5, Resolution- Rs> 2, Tailing factor- As ≤ 2, Theoretical plates- N > 2000.

Cabotegravir IUPAC name is (3R,6S)-N-[2,4-difluorophenyl) methyl]-10-hydroxy-6-methyl-8,11-dioxo-4-oxa-1,7-diazatricyclo [7.4.0.03,7] trideca-9,12-diene-12-carboxamide. Molecular Formula for the compound cabotegravir is C19H17F2N3O5 and the Molecular Weight is 405.4 g/mol and is known as HIV drug. Soluble in organic solvents such as DMSO and dimethyl formamide. Cabotegravir is an investigational integrase strand transfer inhibitor currently under study for HIV prevention (preexposure prophylaxis) and treatment (in combination with rilpivirine) [4-7]. 022 Oct 4;328(13):1304–1314.

Fig.1: Molecular Structure of Cabotegravir

Rilpivirine, with the IUPAC name 4-[4-[4-[(E)-2-cyanoethenyl]-2,6-dimethylanilino] pyrimidin-2-yl]amino]benzonitrile, has a molecular formula of



$C_{22}H_{18}N_6$ and a molecular weight of 366.16 g/mol. It is soluble in organic solvents like DMSO and DMF. Rilpivirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used as part of combination therapy for HIV infection [8-11].

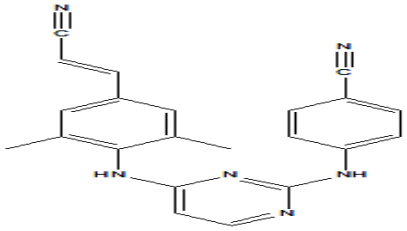


Fig.2: Molecular structure of Rilpivirine

Materials and Methods

Chemicals and Reagents

Acetonitrile- HPLC grade, Formic acid- HPLC, Ammonium formate- HPLC, Water (Milli Q)- HPLC, Cabotegravir- GlaxoSmithKline Mercury Limited. Rilpivirine- Janssen-Cilag Spa, Limited.

Instruments and Apparatus required

High performance liquid chromatography system-Waters e 2695- Empower software 2.0versions (ALLIANCE) with UV/VIS spectrophotometer detector. pH meter is Eutech, Pipettes, beakers and Burettes are Borosil, Ultra sonicator is Unichrome.

Instrumentation and Chromatographic condition

The HPLC system (Waters e 2695- Empower software2.0versions) equipped with PDA detector, X-Bridge Phenyl Column (150×4.6mm 3.5µ), was used to achieve chromatographic separation [12-15]. Mobile phase was composed of Acetonitrile and Ammonium formate pH-3.5 adjusted with formic acid is 30:70v/v ratio. Load volume of drug solution was 10µl, and the detection was recorded at 269nm [16-19].

Preparation of Ammonium formate buffer solution

0.315g of Ammonium formate dissolved in 1Ltr HPLC water pH-3.5 adjusted with Formic acid. Filter through 0.45µ nylon filter [20-21].

Preparation of Mobile Phase: Mobile phase was prepared by mixing Ammonium formate pH-3.5/Formic acid and ACN taken in the ratio 70:30. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram. Mobile Phase was used as a diluent.

Standard Solution Preparation

20 mg of Cabotegravir, 30 mg of Rilpivirine working standards were accurately weighed and transferred into a 10 ml cleaned dry volumetric flask Diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent [21-24]. (Stock solution). Further 1 ml of the above stock solutions was pipetted out into a 10 ml volumetric flask and diluted up to the mark with diluent. (200 mg/mL of Cabotegravir, 300 mg/mL of Rilpivirine).

Determination of Working Wavelength (λmax)

The wavelength of maximum absorption of the solution of the drugs in mixture of Acetonitrile and Ammonium formate pH-3.5/formic acid (30:70) were scanned using PDA detector within the wavelength region of 200–400 nm against Acetonitrile and Ammonium formate pH-3.5/formic acid (30:70) as blank. The absorption curve shows isobestic point at 269nm.

Method validation

System Suitability

Tab.1: System suitability parameters for Cabotegravir and Rilpivirine

S.No	Parameter	Cabotegravir	Rilpivirine
1.	Retention time	2.018	3.465
2.	Plate count	2112	5166
3.	Tailing factor	1.07	1.01
4.	Resolution	--	7.78
5.	%RSD	0.17	0.36

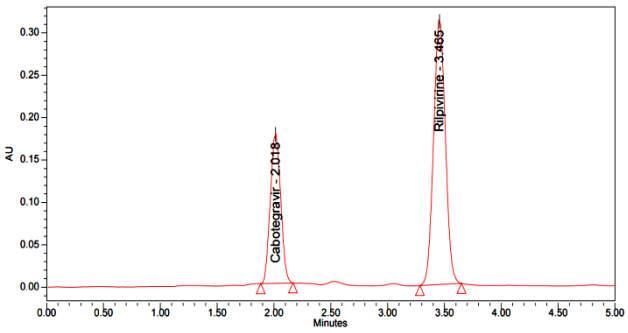


Fig:3 System suitability

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2.

Specificity

Discussion: Retention times of Cabotegravir and Rilpivirine were 2.014 min and 3.455 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

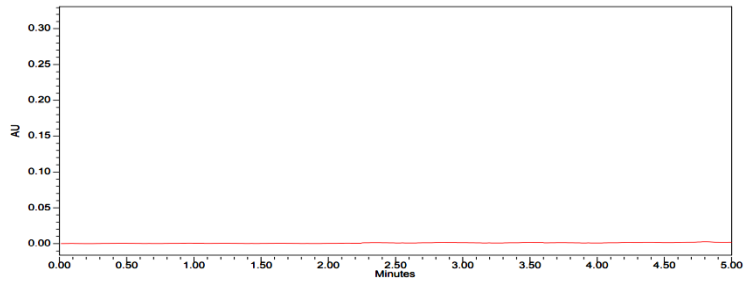


Fig.4: Chromatogram of blank

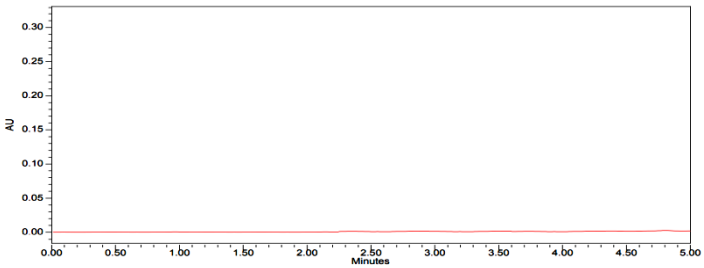


Fig.5: Chromatogram of placebo

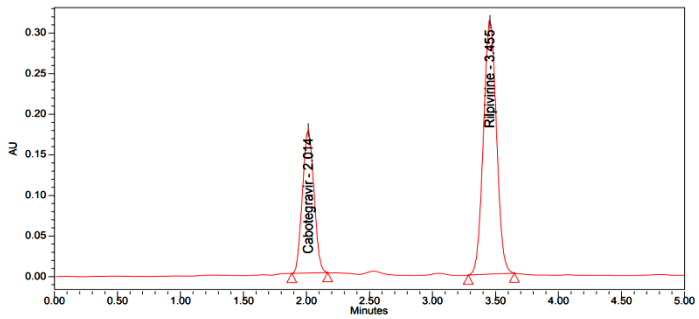


Fig.6: Optimized chromatogram

Linearity

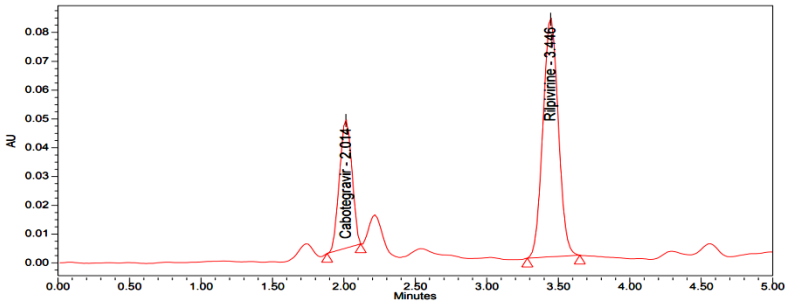


Fig.7: Chromatogram of Linearity-25%

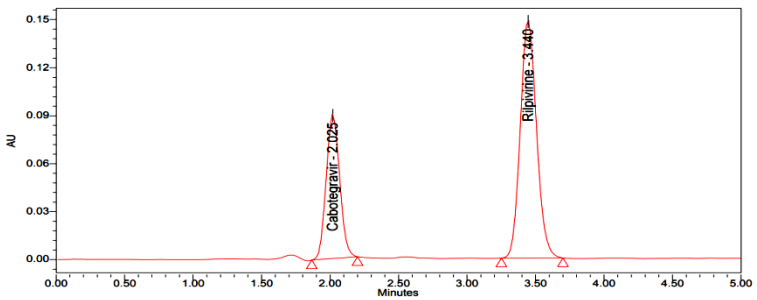


Fig.8: Chromatogram of Linearity-50%

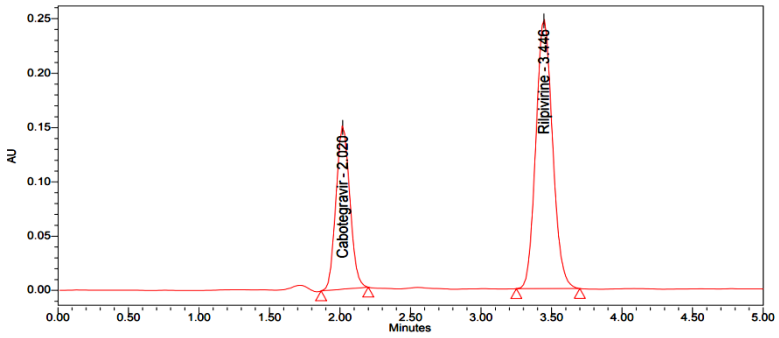


Fig.9: Chromatogram of Linearity-75%

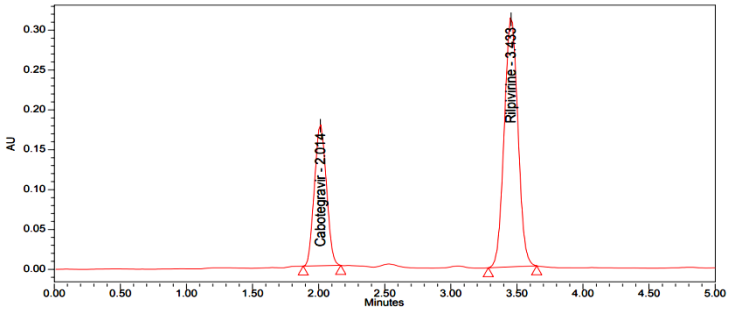


Fig.10: Chromatogram of Linearity-100%

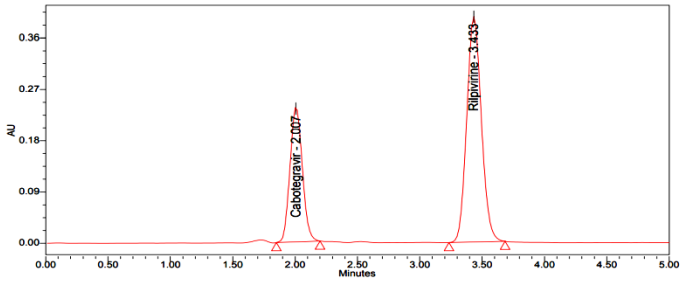


Fig.11: Chromatogram of Linearity-125%

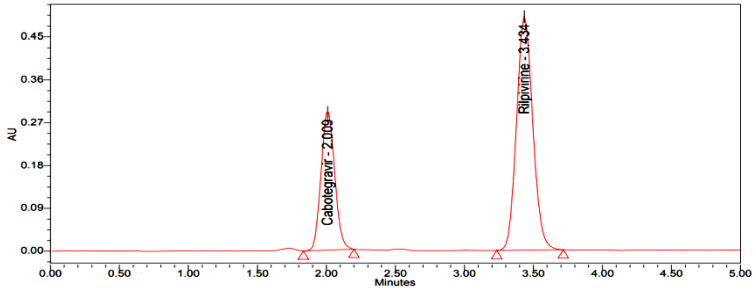


Fig.12: Chromatogram of Linearity-150%

Tab.2: Results of linearity for Cabotegravir and Rilpivirine

S.No	Cabotegravir		Rilpivirine	
	Conc.(µg/mL)	Peak area	Conc.(µg/mL)	Peak area
1	50.00	572659	75.00	767459
2	100.00	1084453	150.00	1587563
3	150.00	1645982	225.00	2343598
4	200.00	2182698	300.00	3068982
5	250.00	2644593	375.00	3737967

6	300.00	3161526	450.00	4530639
Regression equation	y = 10378x +65838		y = 9901.9x +73452	
Slope	10378		9901.9	
Intercept	65838		73452	
R^2	0.9993		0.9992	

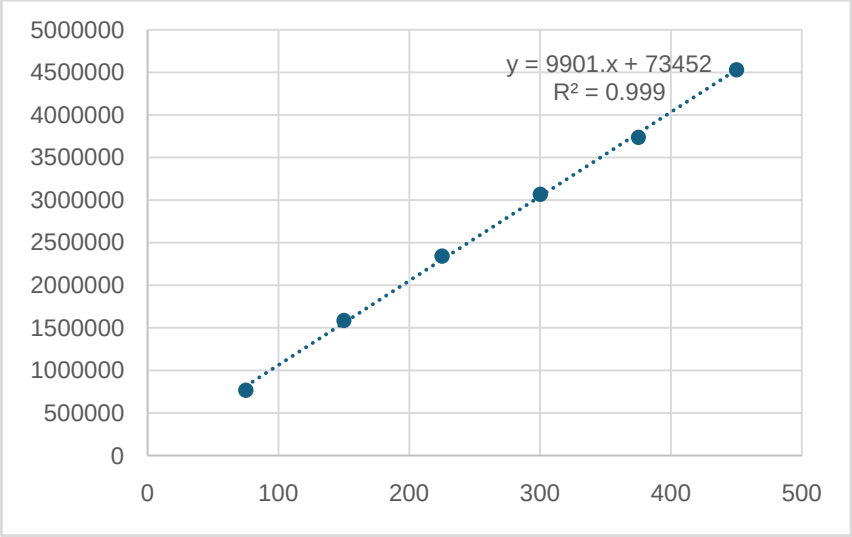


Fig.13: Calibration curve for Rilpivirine at 269 nm

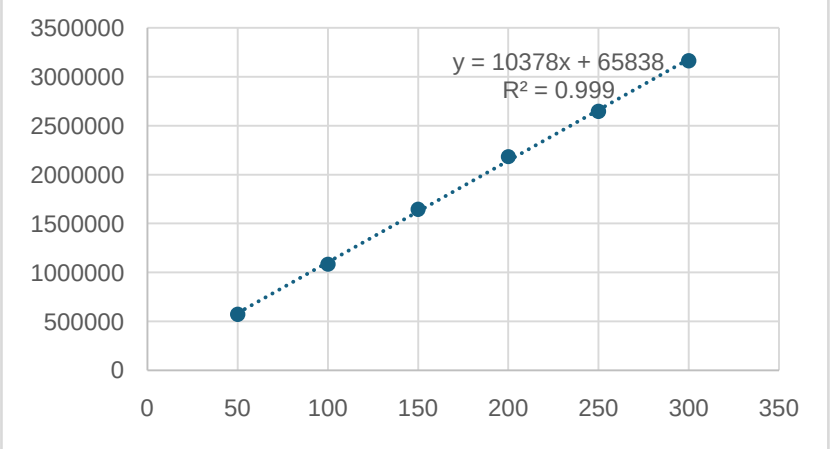


Fig.14: Calibration curve for Cabotegravir at 269 nm

Accuracy

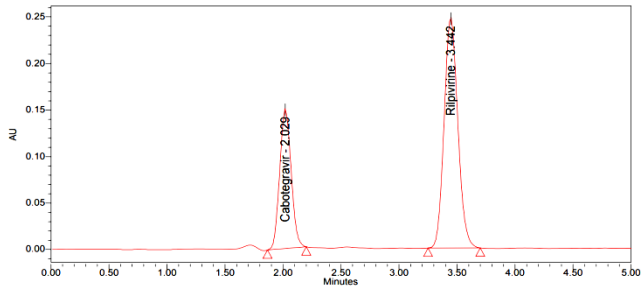


Fig.15: Chromatogram of Accuracy 80%

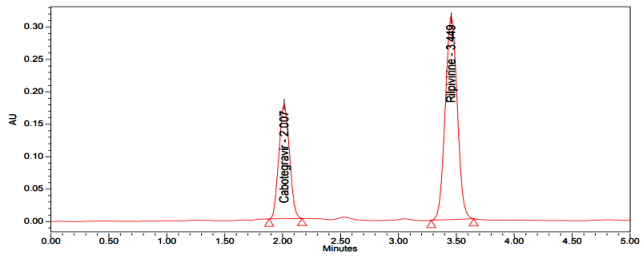


Fig.16: Chromatogram of Accuracy 100%

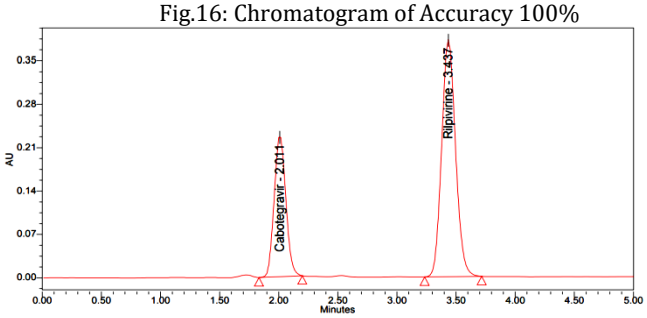


Fig.17: Chromatogram of Accuracy 120%

Tab.3: Accuracy results of Cabotegravir by RP-HPLC method

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	1671482	16	15.79	98.7	99.7%
100%	2111742	20	19.95	99.8	
120%	2552871	24	24.12	100.5	

Tab.4: The Accuracy results for Rilpivirine by RP-HPLC method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	2417441	24	23.94	99.8	100.3%
100%	3045921	30	30.16	100.5	
120%	3652308	36	36.17	100.5	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.7% and 100.3% for Cabotegravir and Rilpivirine respectively.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%

Precision

Precision is the degree of repeatability of an analytical method under normal operation conditions. Precision is of 3 types

1. System precision
2. Method precision
3. Intermediate precision (a. intraday precision, b. Inter day precision)

System Precision

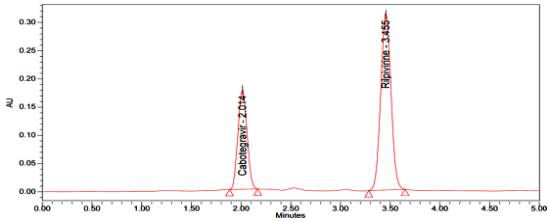


Fig.18: System precision chromatogram-1

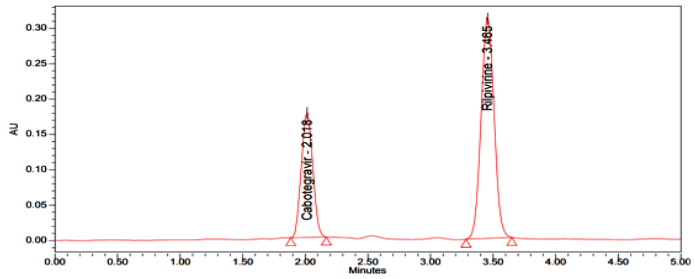


Fig.19: System precision chromatogram-2

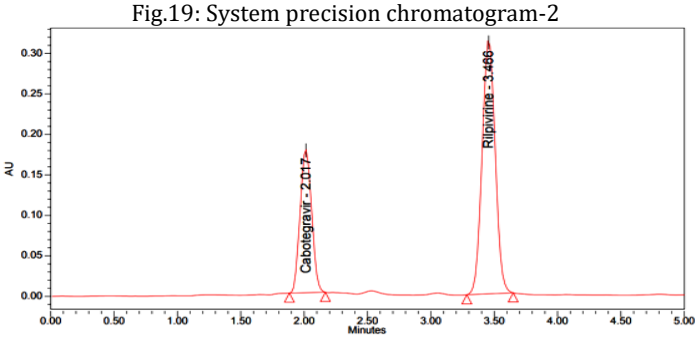


Fig.20: System precision chromatogram-3

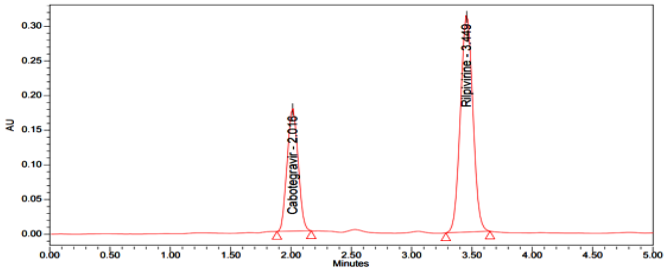


Fig.21:System precision chromatogram-4

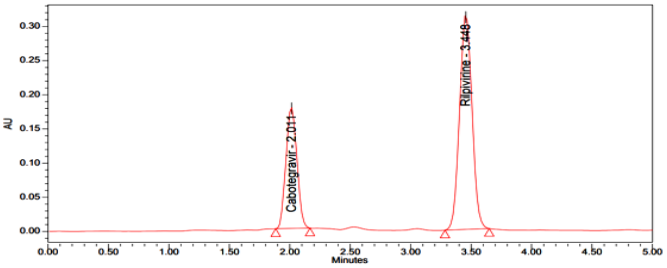


Fig.22: System precision chromatogram-5

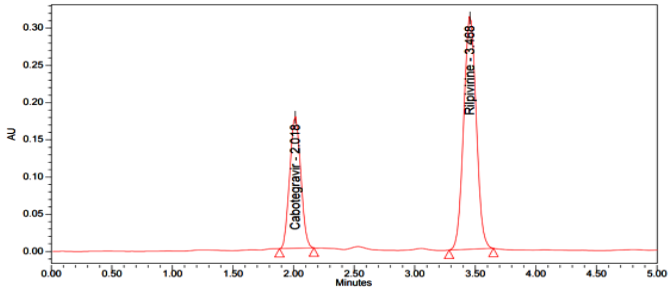


Fig.23: System precision chromatogram-6

Tab.5: System precision table of Cabotegravir and Rilpivirine

S. No	Concentration Cabotegravir (µg/mL)	Area of Cabotegravir	Concentration of Rilpivirine (µg/mL)	Area of Rilpivirine
1.	200	2120286	300	3025038
2.	200	2113277	300	3024948

3.	200	2120624	300	3033624
4.	200	2118056	300	3046612
5.	200	2111947	300	3031321
6.	200	2118119	300	3014352
Mean	-	2117052	-	3029316
S.D	-	3624.25	-	10798.45
%RSD	-	0.17	-	0.36

Discussion: 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 0.17% and 0.36% respectively for Cabotegravir and Rilpivirine. As the limit of Precision was less than "2" the system precision was passed in this method.

Method precision

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

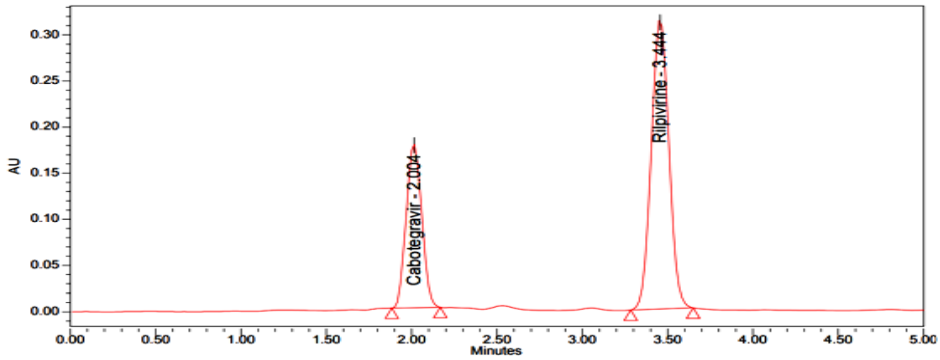


Fig.25: Representation chromatogram for Inter day precision.

Tab.7: Intermediate Precision (Day variation) for Cabotegravir and Rilpivirine by RP-HPLC method

S. No.	Area for Cabotegravir		Area for Rilpivirine	
	Day-1	Day-2	Day-1	Day-2
1.	2118125	2153179	3024786	3042651
2.	2114871	2120252	3072367	3070623
3.	2127154	2147194	3041542	3053964
4.	2116245	2131490	3035143	3062977
5.	2110871	2146512	3046357	3038108
6.	2127652	2135420	3012687	3057165
Average	2119153	2139008	3038814	3054248
Standard Deviation	6821.866	12209.172	20410.898	12230.418
%RSD	0.32	0.57	0.67	0.40

Discussion

Six injections of working standard solution were given on two days and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for both drugs. % RSD obtained as 0.32% and 0.67% respectively for Cabotegravir and Rilpivirine. As the limit of Precision was less than "2" the system precision cabotegravir and rilpivirin was 0.17% and 0.36%.

Acceptance Criteria

The % RSD for the absorbance of six replicate injections results should not be more than 2%.

Robustness

Tab.8: Robustness results of Cabotegravir by RP-HPLC

Parameter	Cabotegravir				
	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change(mL/min)	Less flow(0.8mL)	2.245	2428564	1.11	2239
	Actual(1mL)	2.014	2120286	1.05	2106
	More flow(1.2mL)	1.839	1846481	1.02	2065
Organic Phase change	Less Org (27:73)	2.024	2659501	1.15	2277
	Actual(30:70)	2.018	2113277	1.07	2112
	More Org(33:67)	2.035	1765248	1.04	2034

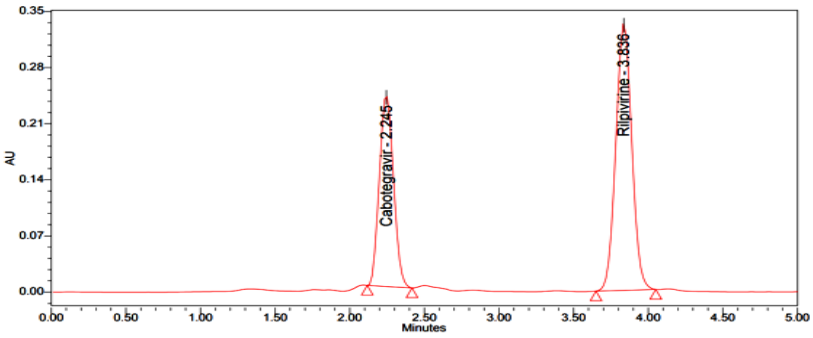


Fig.26: Chromatogram for less flow rate (0.8 mL)

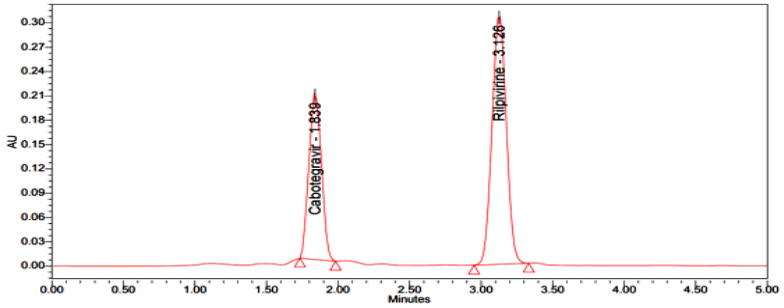


Fig.27: Chromatogram for more flow rate (1.2mL)

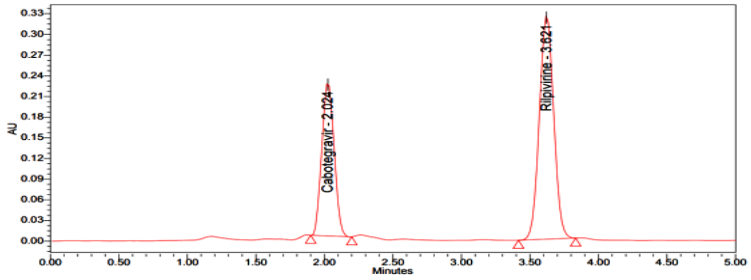


Fig.28: Chromatogram for less Organic Phase (27:73)

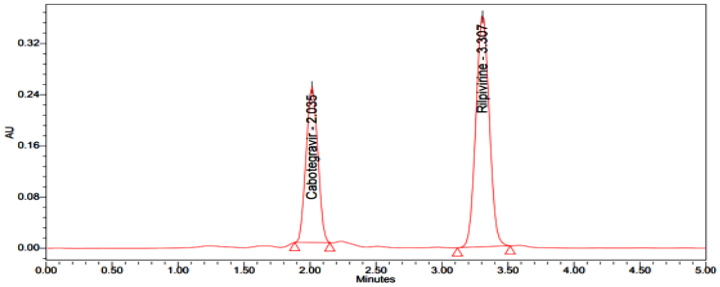


Fig.29: Chromatogram for more Organic Phase (33:67)

Tab.9: Robustness results of Rilpivirine by RP-HPLC

Parameter	Rilpivirine					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change(mLin)	Less flow(0.8mL)	3.836	3365623	8.32	1.10	5199
	Actual(1mL)	3.455	3025038	7.74	1.03	5145
	More flow(1.2mL)	3.126	2966298	7.16	1.00	5086
Organic Phase change	Less Org (27:73)	3.621	3557542	8.58	1.14	5242
	Actual(30:70)	3.465	3024948	7.77	1.06	5137
	More Org(33:67)	3.307	2688502	7.07	1.02	5043

LOD and LOQ (µg/mL)

Tab.10: Sensitivity parameters (LOD & LOQ) by RP-HPLC

Name of drug	LOD(µg/mL)	LOQ(µg/mL)
Cabotegravir	6	20
Rilpivirine	9	30

A simple, efficient, and less time-consuming RP-HPLC method for simultaneous estimation of cabotegravir and Rilpivirin in tablet dosage form was developed and validated for Specificity, Linearity and Range, Accuracy, System Precision, Method Precision, Intermediate Precision, Robustness, LOD and LOQ. The results obtained were good and found within the limit, proving that the developed method can be used for determination of cabotegravir and Rilpivirin tablets.

Conclusion

A stability indicating RP- HPLC method was developed for the simultaneous estimation of the Cabotegravir and Rilpivirine by using mobile phase containing Ammonium formate pH-3.5/formic acid & ACN in the ratio of 70:30% v/v, Waters X-Bridge phenyl column (150x4.6mm, 3.5µm). The flow rate was 1.0 mL/min. Detection was carried out by absorption at 269nm. Retention time of Cabotegravir and Rilpivirine were found to be 2.014min and 3.455min. % RSD of system precision for Cabotegravir and Rilpivirine were found to be 0.17 and 0.36 respectively, % RSD of method precision for Cabotegravir and Rilpivirine were found to be 0.38 and 0.57 respectively. % Recovery was obtained in the range of 99.7% and 100.3% for Cabotegravir and Rilpivirine respectively. Regression equation of Cabotegravir is y =10378x+65838, and Rilpivirine is y=9901x+73452. LOD, LOQ values obtained from regression equations of Cabotegravir and Rilpivirine were 6µg/ml, 9µg/ml. and 20µg/mL, 30µg/ml. respectively. Regression coefficient for Cabotegravir and Rilpivirine were found to be 0.9993 and 0.9992 respectively. The developed HPLC method for the estimation of selected drugs is simple, rapid, accurate, precise, robust and economical and has no interference with the placebo and degradation products. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

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Author Contribution

All are contributed equally

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