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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CABOTEGRAVIR & RILPIVIRINE BY RP HPLC METHOD

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Abstract:
A new method was established for simultaneous estimation of and cabotegravir and rilpivirine by RP-HPLC method. The Phosphate buffer was pH 3.2 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 40:60 % v/ v. A Inertsil ODS C18 (4.6 x 250mm, 5 µm, Make Waters) column used as stationary phase. The detection was carried out using UV detector at 231 nm. The solutions were obtained at a constant flow rate of 1.0 ml/min. The linearity range of cabotegravir and rilpivirine were found to be from 20-60 g/ml and 30-90 g/ml respectively. linear regression coefficient was not more than 0. 999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Cabotegravir and Rilpivirine LOD and LOQ was found to be within limit.

Key words: *Cabotegravir and Rilpivirine, RP-HPLC, Acetonitrile.*

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INTRODUCTION:

Cabotegravir is an HIV-1 integrase inhibitor used for treatment and pre-exposure prophylaxis of HIV-1 infection. Oral cabotegravir is indicated in combination with rilpivirine for the short-term treatment of HIV-1 in virologically suppressed adults with no history of treatment failure to assess tolerability of cabotegravir or who have missed an injected dose of cabotegravir. [1] Intramuscular extended-release cabotegravir in combination with rilpivirine is indicated as a complete regimen for the treatment of HIV-1 infection in adults and adolescents 12 years of age and older weighing at least 35 kg to replace the current antiretroviral regimen in those who are virologically suppressed (HIV-1 RNA <50 copies/mL) on a stable antiretroviral regimen with no history of treatment failure and with no known or suspected resistance to either cabotegravir or rilpivirine. [2] 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. IUPAC name of Cabotegravir is N-[(2,4-difluorophenyl) methyl]-10-hydroxy-6-methyl-8,11-dioxo-4-oxa-1,7-diazatricyclo [7.4.0.0^ {3,7}]trideca-9,12-diene-12-carboxamide. Molecular Formula is $C_{19}H_{17}F_2N_3O_5$. Molecular Weight is 405.3.

Rilpivirine, in combination with other agents, is indicated for the treatment of HIV-1 infections in antiretroviral treatment-naive patients with HIV-1 RNA ≤100,000 copies/mL and CD4+ cell count >200 cells/mm. [3] The FDA combination therapy approval of rilpivirine and dolutegravir is indicated for adults and adolescents 12 years of age and older weighing at least 35 kg with HIV-1 infections whose virus is currently suppressed (< 50 copies/ml) on a stable regimen for at least six months, without a history of treatment failure and no known substitutions associated to resistance to any of the two components of the therapy. [4] Rilpivirine is a non-competitive NNRTI that binds to reverse transcriptase. Its binding results in the blockage of RNA and DNA- dependent DNA polymerase activities, like HIV-1 replication.[5] It does not present activity against human DNA polymerases α, β and γ. IUPAC name of Rilpivirine is 4-{[4-({4-[(1E)-2-cyanoeth-1-en-1-yl]-2,6-dimethylphenyl}amino)pyrimidin-2-yl]amino}benzonitrile. Molecular formula is $C_{12}H_{18}N_6$. Molecular Weight is 366.4.

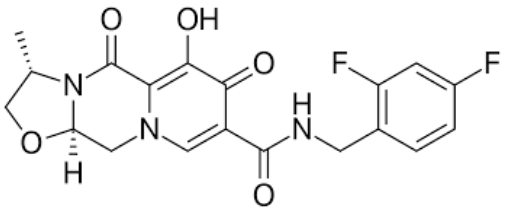


Figure 1: Structure of Cabotegravir

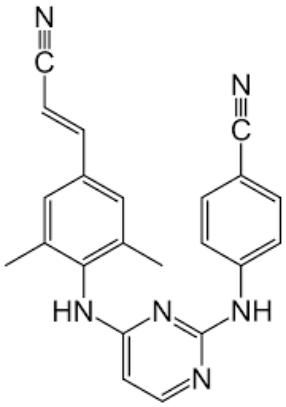


Figure 2: Structure of Rilpivirine

Literature survey shows that a number of methods have been reported for estimation of Cabotegravir And Rilpivirine individually or in combination with other drugs.[6-10] Present study aims to develop simple, rapid, greater sensitivity and faster elution by using RP-HPLC for the simultaneous estimation of Cabotegravir & Rilpivirine. The developed RP-HPLC method was applied for the forced degradation studies and the method was validated for its parameters as per ICH guidelines.

MATERIALS AND METHODS:

Chemicals and Reagents:

Cabotegravir and Rilpivirine were gifted samples. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 231 nm with column (4.6 x 250mm, 5µm), Make: Inertsil ODS, dimensions at 25^0C temperature. The optimized mobile phase consists of

phosphate Buffer: acetonitrile (60: 40 v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of Phosphate buffer

Accurately weighed 6.5 grams of potassium dihydrogen ortho phosphate and placed into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.2 with Orthophosphoric acid.

Preparation of mobile phase

Accurately measured 600 ml (60%) of above buffer and400 ml of Acetonitrile HPLC (40%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.22 µ filter under vacuum filtration and used as diluent.

Standard Solution Preparation

Weighed 200mg of Cabotegravir and 300mg Rilpivirine into 500ml volumetric flask and diluted with 70 ml of diluent and sonicated. made upto volume mark with diluent.Taken 5.0ml from above stock solution into 50ml flask and diluted to mark with diluent to get final concentration of40µg/ml of cabotegravir and 60 µg/ml of rilpivirine

Sample Solution Preparation

Taken equivalent volume of injection solution such that 200 mg of Cabotegravir and 300 mg Rilpivirine sample were weighed and transferred into a 500 ml clean dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and made upto volume mark with diluent. Further, 5.0 ml of above stock solution was diluted to 50ml with diluent to get final concentration of 40µg/ml of cabotegravir and 60 µg/ml of rilpivirine The % Assay was calculated using the following formula

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Cabotegravir and Rilpivirine peaks and calculate the %Assay by using the formulae**.**

RESULTS AND DISCUSSION:

Method:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into (4.6 x 250mm, 5µm), Make: Inertsil ODS, the mobile phase of composition phosphate Buffer: acetonitrile (60: 40 v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters of Cabotegravir and Rilpivirine

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Rilpivirine**	2.003	920101	112653		1.45	6895.63
2	**Cabotegravir**	5.067	552058	41389	10.57	1.33	7053.66

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Cabotegravir and Rilpivirine in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Cabotegravir and Rilpivirine

S. No	Name of compound	Label claim(mg)	Amount taken(mg)	%Purity
1	Cabotegravir	200	200.01	99.813
2	Rilpivirine	300	300.01	99.910

Figure 3: Standard chromatogram

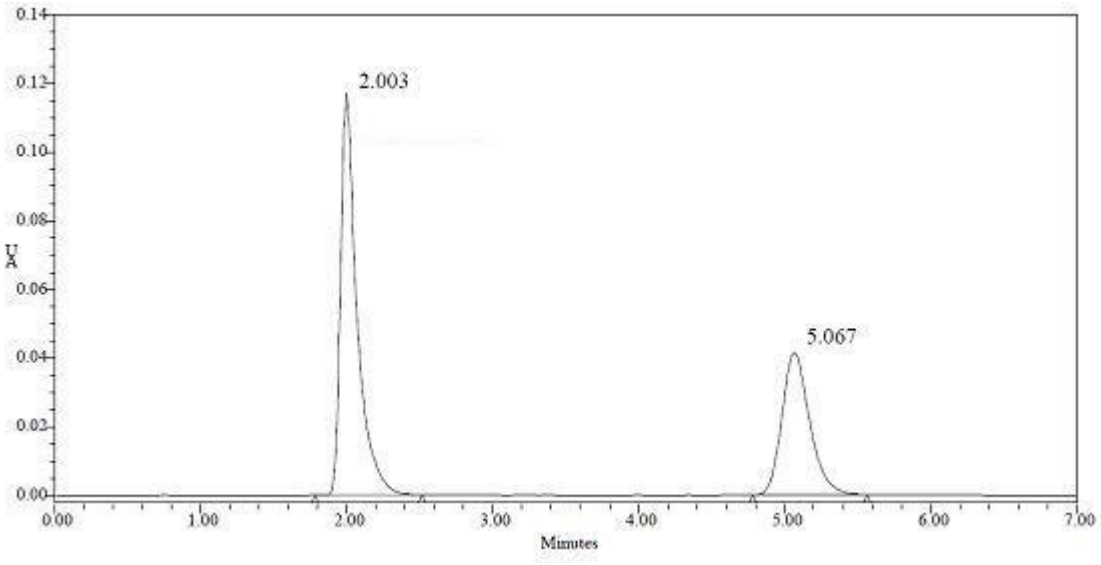
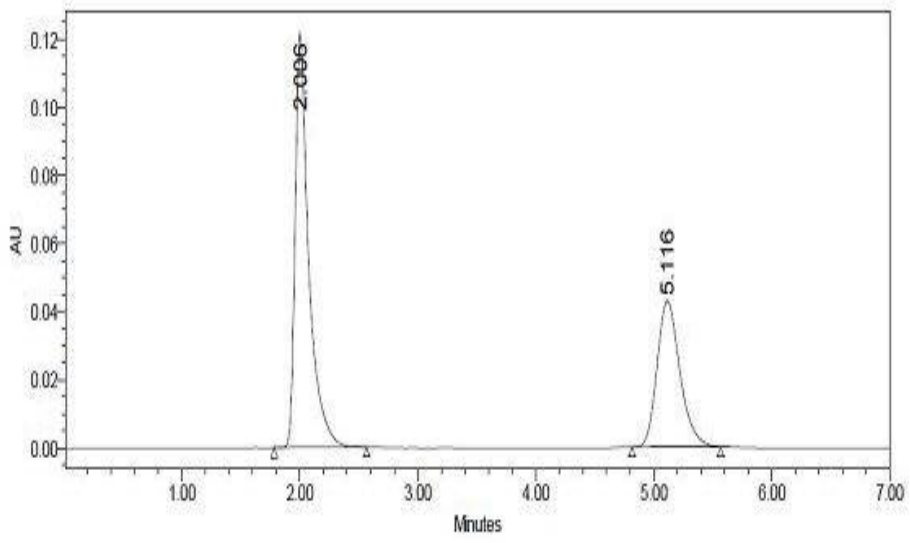


Figure 4: Sample chromatogram



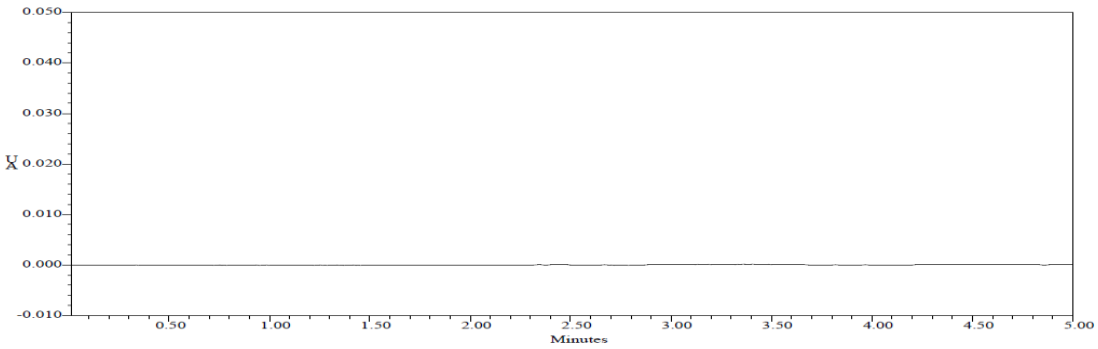


Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 20 µg/ml to 60 µg/ml and 30 µg/ml to 90 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3,4.

Table 3: Linearity results of Cabotegravir

SI.NO	Linearity level	Concentration	Area
1	I	20 ppm	279604
2	II	32ppm	446652
3	III	40ppm	558256
4	IV	48ppm	663145
5	V	60ppm	837109
Correlation Coefficient			0.999

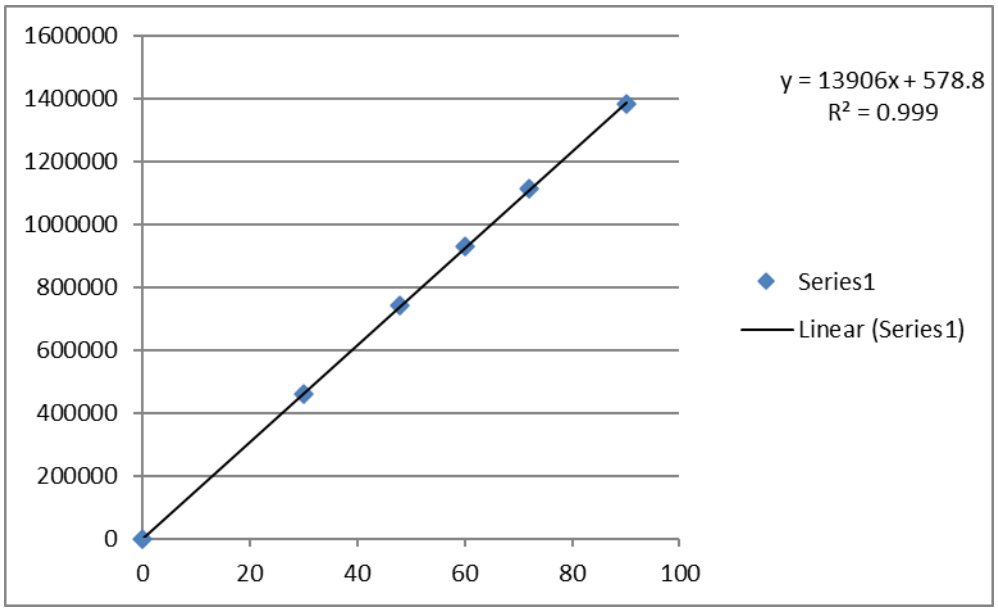


Figure 6: Linearity graph for Cabotegravir

Table 4: Linearity results of Rilpivirine

S.No	Linearity Level	Concentration	Area
1	I	30 ppm	460523
2	II	48 ppm	743569
3	III	60 ppm	929610
4	IV	72 ppm	1115532
5	V	90 ppm	1384575
Correlation Coefficient			0.999

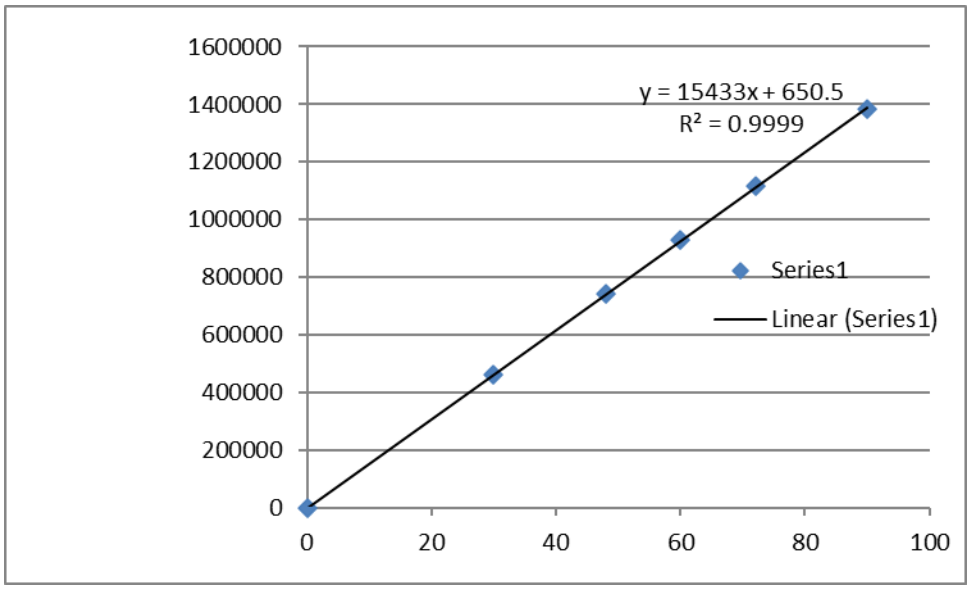


Figure 7: Linearity graph for Rilpivirine

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Cabotegravir and Rilpivirine and calculate the individual recovery and mean recovery values. The results are shown in table 5.

Table 5: Showing accuracy results for Cabotegravir and Rilpivirine

Sample concentration	Sample set no	Sample area		%Assay		% Recovery	
		Rilpivirine	Cabotegravir	Rilpivirine	Cabotegravir	Rilpivirine	Cabotegravir
50%	**1**	464769	279632	49.996	50.065	99.99	100.0
	2	464532	279501	49.971	50.042	99.94	99.6
	3	464009	279008	49.914	49.954	99.83	99.6
	Average Recovery					**99.92%**	**99.73%**
100%	**1**	921562	558695	99.13	100.03	99.13	100.03
	2	923568	558456	99.35	99.99	99.35	99.99
	3	922008	558125	99.18	99.93	99.18	99.93
	Average recovery					**99.22%**	**99.98%**
150%	**1**	1394415	837415	150.00	149.93	100.00	99.95
	2	1396652	837446	150.24	149.94	100.16	99.96
	3	1398624	837956	150.45	150.03	100.30	100.02
	Average recovery					**100.15%**	**99.98%**

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6,7.

Table 6: Results of method precision for Rilpivirine:

S. No	Sample area	Standard area	Percentage purity(%)
1	925869	926785	99.60
2	930564	927458	100.10
3	928475	926389	99.88
4	929562	927556	99.99
5	927459	939862	99.77
Average		**929610**	**99.87**
%RSD		**0.61%**	**0.19%**

Table 7: Results of method precision for Cabotegravir

S. No	Sample area	Standard area	Percentage purity(%)
1	553025	554698	99.01
2	553241	556485	99.05
3	555623	557425	99.48
4	557893	562351	99.89
5	559286	561707	100.13
Average		**558533**	**99.51**
%RSD		**0.59%**	**0.50%**

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on Analyst. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 8,9.

Table 8: Ruggedness results of Rilpivirine

S. No	Sample area	Standard area	Percentage purity(%)
1	925339	926785	99.54
2	928754	927458	99.91
3	929556	926389	99.99
4	930656	927556	100.11
5	929866	939862	100.03
Average		**929610**	**99.92**
%RSD		**0.61%**	**0.22%**

Table 9: Ruggedness results of Cabotegravir

S. No	Sample area	Standard area	Percentage purity(%)
1	553128	554698	99.03
2	556304	556485	99.60
3	558963	557425	100.08
4	558745	562351	100.04
5	560239	561707	100.31
Average		**558533**	**99.81**
%RSD		**0.59%**	**0.51%**

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1 ml/min. The results are shown in table 10,11.

Table 10: Results for effect of variation in flow

S. No	peak area for Less flow (0.9 ml/min)		peak area for More flow (1.1 ml/min)	
	Rilpivirine	**Cabotegravir**	**Rilpivirine**	**Cabotegravir**
1	937895	559125	929586	538960
2	942562	559632	925417	543669
3	948965	562359	923159	545890
4	934578	565398	921339	540061
5	936589	567005	922659	545893
Mean	**940118**	**562704**	**924432**	**542895**
%RSD	**0.61**	**0.62**	**0.35**	**0.60**

Table 11: Results for effect of variation in mobile phase composition

S. No	peak area for Less organic (35%)		Peak area for More organic (45%)	
	Rilpivirine	**Cabotegravir**	**Rilpivirine**	**Cabotegravir**
1	980553	530698	910559	585236
2	974521	531526	916892	583567
3	970226	527005	920072	575016
4	975008	525608	919862	580152
5	969856	530074	915745	585963
Mean	**974033**	**528982**	**916626**	**581987**
%RSD	**0.45**	**0.48**	**0.42**	**0.77**

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 12.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 12: LOD, LOQ of Cabotegravir and Rilpivirine

Drug	LOD	LOQ
Cabotegravir	3.01	10.00
Rilpivirine	3.14	10.05

CONCLUSION:

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Rilpivirine and Cabotegravir in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability.

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