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**A RAPID RP-HPLC STABILITY METHOD DEVELOPMENT &  
VALIDATION FOR ANTIVIRAL HIV DRUGS CABOTEGRAVIR &  
RILPIVIRINE IN I.M. INJECTION**

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**ABSTRACT**

In pharmaceutical analysis for the medicinal agents & dosage forms new sophisticated chromatographic methods are utilized for the quality control purpose. There are necessities for new medicinal agents for the treatment therapy for various diseases. Novel antiviral drugs and their combinations are repeatedly developed & synthesised for the therapy for viral diseases. Consequently it requires new methods for the analysis and quality control testing for these new drugs. In the present method is for analysis of antiviral drugs cabotegravir CAB and rilpivirine RILP in pure api & Intramuscular Injection I.M injectable dosage forms. This method is optimised for analysis, of these two drugs in combined forms for rapid analysis with very less amount of analytes drugs utilized for testing purposes. The concentration range for the linearity is 2.5 to 15 µg/ml for CAB cabotegravir & for Rilpivirine RILP it is 3.75 to 22.5 µg/ml, which is very a smaller amount of drugs are utilised. Wavelength selected for estimation is 242.5nm and column used was Kinetex C-18 column (250mm x 4.6mm, 5 µm id). The Retention-time obtained were 2.14min for CAB & 3.12min for RILP. The R<sup>2</sup> was found to be 0.999 for both drugs. The method is used in analysis of drugs in injection formulations individually & in combined forms. The Stability & forced-degradations studies are carried out in different stress conditions and the impurities & pure drugs, are efficiently detected by the developed HPLC method.

**Keywords: RP-HPLC Stability, Antivirals, Cabotegravir, Rilpivirine, Injection**

## INTRODUCTION

In pharmaceutical analysis for the medicinal agents & dosage forms new sophisticated chromatographic methods are utilized for the quality control purpose. There are necessities for new medicinal agents for the treatment therapy for various diseases. Novel antiviral drugs and their combinations are repeatedly developed & synthesised for the therapy for viral diseases. Even currently the use of the antiviral agents has been extensively used for the management of newer diseases like AIDS, Hepatitis, COVID-19, and many other infectious diseases [1].

The newly developed antimicrobial agents like Cabotegravir CAB [2] & Rilpivirine RILP [3] are been widely applied in therapy for diseases like HIV infections [4, 5] also for hepatitis. In pharmaceutical industries there are different individual methods of analysis for these drugs.

The literature reviews also suggests the individual and other combinational HPLC methods [6, 7, 8] of these drugs, but the methods are for single drugs estimations as well as for the other drug combinations, and not available in combined i.m. injectable dosage form along with stability HPLC method. Hence there is a need for the rapid

testing of these drugs by one single HPLC method, as other methods are for individual estimations as well as in combinations only. This HPLC method is developed in which drugs CAB & RILP are analysed and assayed in combined Injection form. The CAB is HIV Integrase Inhibitor class of drug, while RILP NNRTI Non Nucleoside Reverse Transcriptase Inhibitor [9]. They act by blocking the viral DNA replication in the hosts and are highly potent drugs of the antiviral class [10, 11].

Cabotegravir CAB iupac name is (3 - R, 6 - S) - N - [ (2,4 - di-fluoro-phenyl) - methyl - ] - 10 - hydroxyl - 6 - methyl - 8 - 11-dioxo - 4 - oxa - 1-7- di - aza - tricyclo [7.4.0.0] - tri - deca - 9,12 - diene - 12 - carboxamide and Rilpivirine RILP has, iupac name 4 - [ - [ 4 - [ 4 - [ - 2 - cyano - ethenyl - ]. -2,6 - dimethyl - aniline ] pyrimidin - 2 - yl ] amino ] - benzonitrile , which are used in antiviral therapy [12, 13].

### Chemical Structures of Drugs:

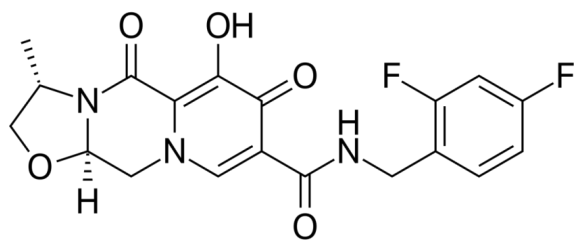


Figure 1: Cabotegravir

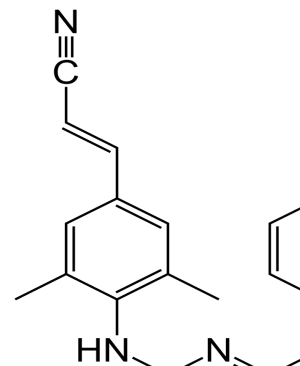


Figure 2: Rilpivirine

## MATERIALS AND METHODS:

### Materials

The Working Reference standards Cabotegravir CAB & Rilpivirine RILP have been obtained from Solesom & Bizotech pharma. The chemicals and reagents Methanol, Acetonitrile, orthophosphoric acid, potassium dihydrogen ortho phosphate, Azobisisobutyronitrile AIBN, has been used analytical grade Merck graded. HCl, NaOH analytical grade of Rankem were used. Milli-Q pure water has been used for sample and mobile phase preparations.

### Instrumentation

Shimadzu HPLC system Class VP 2010 auto sampler & Agilent 1100 both systems has been used for the analysis equipped with both PDA & UV detector. Shimadzu UV 1800 spectrophotometer had been utilized for the wavelength maxima estimation. Wist Temperature Chamber was used for thermal degradation study. Photostability Test

Chamber Sanwood SM-LHH-GSD-UV Series was utilised.

### Chromatographic conditions

The separation of the drugs Cabotegravir CAB & Rilpivirine RILP has been made by using two columns. Kinetex C-18 column & Hypersil ODS (250 mm x 4.6 mm, 5 µm id) for consistent results. The mobile phase is been utilised consists of ratio of % Percentage by volume of Acetonitrile ACN (35) : (65) Sodium Dihydrogen Phosphate buffer (0.05M) of pH 4.5. The flow rate adjusted 1ml/min and detection wavelength was 242.5nm. The column- temperature column oven kept at 25 °C.

### Preparation of Solutions

#### Standard Solutions

The standard solutions of the three drugs working reference standards was prepared at concentrations range of 2.5 to 15 µg/ml for CAB cabotegravir & for Rilpivirine RILP it is 3.75 to 22.5 µg/ml.

### Sample Solution

The sample solutions were prepared from injections vials of the drugs CAB 400mg in 2ml inj vial & RILP 600mg in 2ml vial. And another second type vials contains CAB 600mg in 3ml inj vial & RILP 900mg in 3ml vial. (i.e. each vial type contains CAB 200mg/ml and RILP 300mg/ml). The contents of vials were dissolved in methanol 50 : acetonitrile 50 v/v ratio, sonicated, centrifuged & filtered off to give stock solutions of CAB & RILP. The final dilutions were prepared with mobile phase as 2.5 to 15 µg/ml that is 2.5, 5, 7.5, 10, 12.5, 15 µg/ml for CAB cabotegravir & for Rilpivirine RILP it is 3.75 to 22.5µg/ml that is 3.75, 7.5, 11.25, 15, 18.75, 22.5 µg/ml and used for linearity ranges.

### Method Validation

The analytical method is developed, for drugs Cabotegravir CAB & Rilpivirine RILP is been validated ICH guideline [14] in the terms of precision linearity accuracy LOD LOQ which are required to justify the purpose of the developed method. By employing the use of two columns Kinetex C-18 column & Hypersil ODS (250 mm x 4.6 mm, 5 µm id) for the separation and resolution of these drugs has been efficiently made. The developed analytical method proves to be more quick rapid and efficient

on the basis of the validation as per the ICH guideline protocols.

### System Suitability

The RP-HPLC method is a very accurate and precise method following the system suitability parameters as per the USP and ICH protocols. The system suitability has been justified from the data of the retention time is 2.14min for CAB & 3.12min for RILP of the drugs as well as on the basis of the tailing factors of the chromatograms and from the values of the theoretical plates can be treated as accurate and efficient for the analysis purposes. System suitability parameters are summarised in results discussions **Table 1**.

### Specificity

The chromatogram of the standard reference drugs it was found to be accurate for the individual drug analysis as well as in the combination, and no other impurity or other analyte found to be overlapped in the chromatogram. It was confirmed by individual drug injecting multiple times to confirm that method is highly specific for analysis. Also the placebo blank was injected individually for the sample analysis and it does not interfere in the chromatogram

### Figure 4.

### Linearity

The linearity of the drug response chromatogram **Figure 5** shows that variable concentration has been found to be at the range of 2.5 to 15 µg/ml for CAB that is 2.5, 5, 7.5, 10, 12.5, 15 µg/ml and for RILP is 3.75 to 22.5µg/ml that is 3.75, 7.5, 11.25, 15, 18.75, 22.5 µg/ml mentioned in **Table 3**. The  $R^2$  of both drugs was found to be 0.9999. The drugs CAB & RILP were individually & together optimised, so as to efficiently reduce the cost of the utilization of the working standard analyte drugs.

### Forced Degradation Studies

The HPLC method is moreover applicable in the stability study [15] during forced-degradation stress studies of the drug products and drug substances. It was carried out by ICH Q1 AR2 guidelines in which the degradation of these drug substances was been made out, - by different- stress conditions like Acid, Alkali, Hydrolytic, Thermal and Photo degradation for the drugs substances [16]. In the forced degradation study the 0.1N HCl was utilised and the drug samples were subjected for degradation for at different time intervals of 60, 120 minutes and also on a thermostat heat bath at 60<sup>0</sup>C temperature to accelerate the degradation process. Further Degradation was also carried out at 1N HCl, 2N HCl to analyse higher rate of degradations. Identical process

was carried out in alkaline degradation by using 0.1N NaOH at 60, 120min time intervals and higher rate degraded by using 1N NaOH & 2N NaOH. The control and the samples were neutralized and then analyzed by the developed HPLC method,

The Thermal degradation was carried out in a controlled oven Wist Temperature Chamber at different ranges of 60, 80, 100 <sup>0</sup>C, and the samples were analysed at time intervals of 60mins and at longer durations 120mins, 180mins to 5hrs for analyzing higher degradation rates.

Oxidative stress was applied by using 3% H<sub>2</sub>O<sub>2</sub> and parallel with 0.01M Azobisisobutyronitrile AIBN for the degradation of the drug samples for 60,120 minutes has been done.

Photo UV stress has been applied by putting the drug samples in Photostability Test Chamber Sanwood SM-LHH-GSD-UV Series , Photolytic (1.2million lux hrs and 200watt hrs), the time interval was 6hrs, 12hrs, 24hrs and 48hrs to check the degradation pattern in longer duration of time. The samples and standard of different degradation methods were filtered through nylon membrane 0.45µm and injected individually as well as in the combined forms. The developed HPLC method efficiently detects the drug samples in the

chromatograms **Figure 6, 7, 8, 9** and can be compared with the reference standard chromatograms and % drug recovered in **Table 5**.

The major peaks of individual drugs are unaffected by impurity peaks, and can be efficiently resolved with peak purity analysis in **Figures 10, 11 & Table 4** shows that justifies no interference, merging or overlapping of other peaks.

#### **Precision**

The developed method is validated and has been marked precise as per the validation parameter performed in this method. Different samples and standards in six sample and standards were injected multiple times and the SD and RSD was determined. The assay results were also performed and checked for each drug. Data shows the how the method is precise in **Table 6**.

#### **Accuracy**

The accuracy studies- have been carried out as per the guideline at three different levels. The level of 50, 100 and 150 % has been made,- for justifies accuracy for developed method. For the api pure standard and the test samples the accuracy study had been done for showing accurate performance of the developed method shown in **Table 7**.

#### **Assay of Marketed Formulations:**

The foremost purpose of this method is to develop a quick & specific single assay,- method for the i.m. injections in vials. Each vial contains CAB 200mg/ml and RILP 300mg/ml. CABENUVA<sup>TM</sup> (Cabotegravir 200 mg/1ml & Rilpivirine 300 mg/1ml) ER-Injection. CABENUVA 400-mg/600-mg Kit ER-Injection: consists of single dose vial of 400 mg in 2ml (200 mg/1ml) cabotegravir & a single dose vial of 600 mg in 2 ml (300 mg/1ml) rilpivirine.

CABENUVA 600-mg/900-mg Kit ER-Injection: consists of single dose vial of 600 mg in 3ml (200 mg/1ml) cabotegravir & a single dose vial of 900 mg in 3ml (300 mg/1ml) Rilpivirine. The assay method is developed for both the Kits, individually & in combination of CAB 200mg + 300mg RILP. Assay results are shown in the **Table 8 & 9**.

#### **RESULTS & DISCUSSIONS:**

The New Rapid; Precise; Accurate, RP-HPLC method is been successfully developed for the Cabotegravir and Rilpivirine within a very short run time within 5 minutes & the drugs can be qualitatively and quantitatively analysed along with the stability studies.

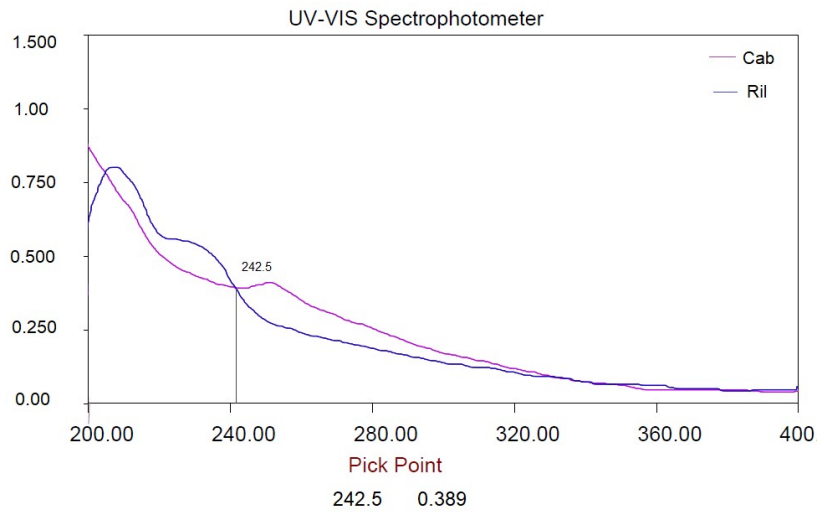


Figure 3: UV overlay Spectra of CAB & RILP 242.5nm wavelength for estimation

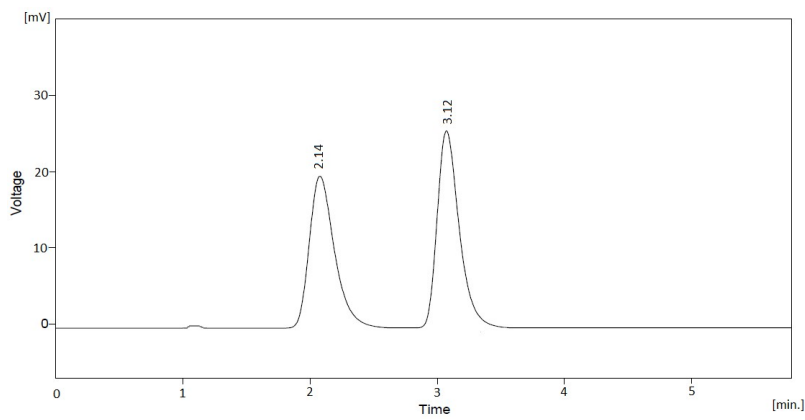


Figure 4: Chromatogram Standard CAB 10µg/ml & RILP 15µg/ml

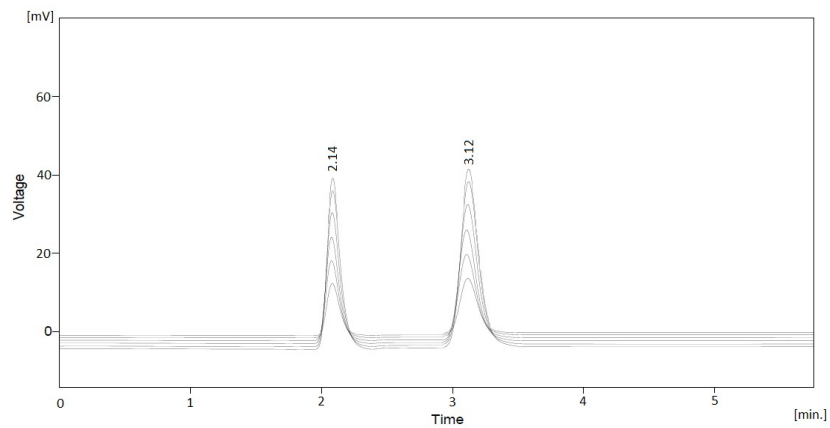


Figure 5: Overlay Linearity CAB 2.5 to 15µg/ml, RILP 3.75 to 22.5µg/ml

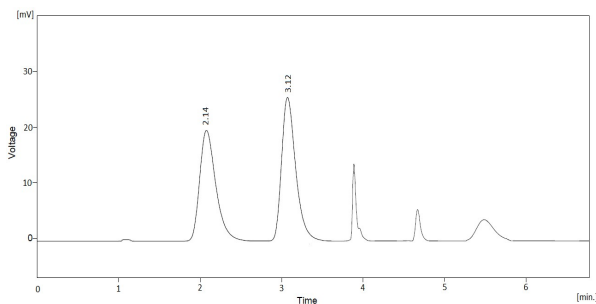


Figure 6: Acid Degradation

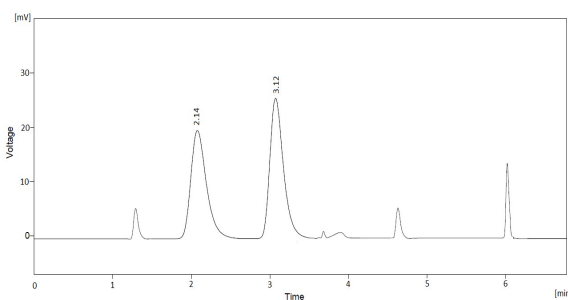


Figure 7: Base Degradation

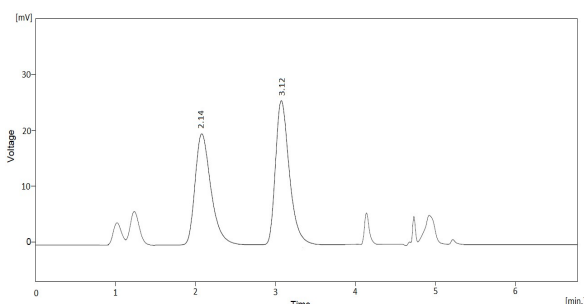


Figure 8: Thermal Degradation

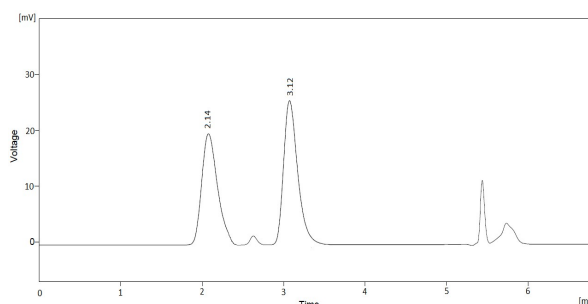


Figure 9: Oxidative Degradation

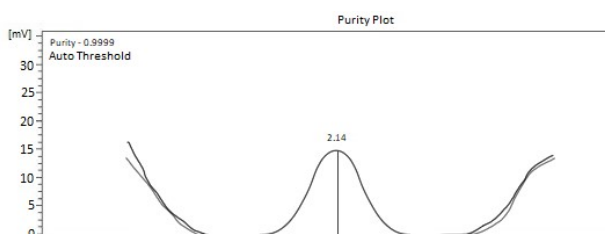


Figure 10: Peak Purity of CAB

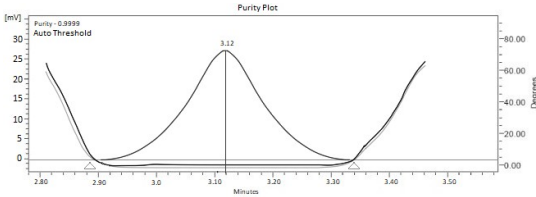


Figure 11: Peak Purity of RILP

Table 1: Summarized Results for the Developed HPLC Method

Serial No	Parameters	Criteria of Acceptance	Results
1	System Suitability	Theoretical Plates NLT 2000	CAB- 15412 RILP- 17644
		Tailing Factor NMT 2.0	CAB- 1.12 RILP- 1.14
		Retention Time Min	LAM- 2.14 RILP- 3.12
2	Precision	% RSD NMT 2.0	CAB- 0.74 RILP- 0.58
3	Linearity	Correlation Coefficient NLT 0.999	LAM- 0.9999 RILP- 0.9999
4	Accuracy	% Recovery 98-102%	CAB- 99.44 RILP- 100.23
5	LOD	1:3 ( Conc. in µg/ml )	CAB- 0.263 RILP- 0.202
6	LOQ	1:10 ( Conc. in µg/ml )	CAB- 0.798 RILP- 0.613
7	Assay	% Label Claim	CAB- 99.80 RILP- 99.95



Table 2: Optimised Chromatographic Conditions

Parameters	Chromatographic Conditions
Mobile Phase Ratio	Acetonitrile ACN (35) : (65) Sodium Dihydrogen Phosphate buffer (0.05M) of pH 4.5
Column	Kinetex C-18 column & Hypersil ODS ( 250 mm x 4.6 mm, 5 µm id )
Detector	PDA & UV
Column Temp	25 °C
Wavelength	242.5nm
Flow Rate	1ml/min
Injection Volume	2µl

Table 3: Linearity Data of CAB &amp; RILP

Linearity	CAB		RILP	
	Conc. µg/ml	Area	Conc. µg/ml	Area
1	2.5	561.47	3.75	612.75
2	5	1126.34	7.5	1235.87
3	7.5	1722.36	11.25	1840.25
4	10	2250.33	15	2451.298
5	12.5	2812.21	18.75	3089.19
6	15	3385.31	22.5	3675.46

Table 4: Stress Degradation Peak Purity Data

	PEAK PURITY ANGLE		PEAK PURITY THRESHOLD		PEAK PURITY	
	CAB	RILP	CAB	RILP	CAB	RILP
Acid	0.113	0.123	0.295	0.298	0.999	0.999
Base	0.134	0.134	0.316	0.314	0.997	0.998
Oxidative	0.139	0.133	0.346	0.366	0.998	0.999
Thermal	0.126	0.136	0.311	0.297	0.999	0.998
Photolytic	0.134	0.129	0.321	0.388	0.999	0.998
Hydrolytic	0.137	0.211	0.346	0.347	0.998	0.999

Table 5: Stress Degradation study summarized data

Degradation Condition	Peak Area		% Drug Recovered		% Degraded	
	CAB	RILP	CAB	RILP	CAB	RILP
Acid	2248.32	2249.36	99.90	99.92	0.08	0.07
Base	2238.23	2438.32	99.46	99.47	0.53	0.52
Oxidative	2233.02	2428.23	99.23	99.05	0.76	0.94
Thermal	2232.12	2437.66	99.19	99.44	0.80	0.55
Photolytic	2229.32	2433.25	99.06	99.26	0.93	0.73
Hydrolytic	2248.22	2430.21	99.09	99.13	0.09	0.86

Table 6: Precision Repeatability Data

Number of Injections	CAB Area 10 µg/ml	RILP Area 15 µg/ml
1	2250.33	2451.29
2	2252.46	2452.12
3	2255.31	2455.32
4	2253.88	2459.67
5	2255.98	2454.41
6	2253.44	2449.22
AVG	2253.56	2453.67
SD	2.03	3.66
% RSD	0.09	0.14

Table 7: Accuracy Data at Three different levels

DRUG	Sample Amount $\mu\text{g/ml}$	% Conc. Level	Total Area	Net Area	Amount STD Added $\mu\text{g/ml}$	Amount STD Recovered $\mu\text{g/ml}$	% Recovery	Mean Recovery
CAB	10	50	3350.66	1100.32	5	4.88	97.79	99.44
		100	4513.45	2263.11	10	10.05	100.56	
		150	5624.98	3374.64	15	14.99	99.97	
RILP	15	50	3679.21	1227.91	7.5	7.51	100.18	100.23
		100	4910.24	2458.94	15	15.04	100.31	
		150	6135.65	3684.35	22.5	22.54	100.20	

Table 8: Assay of Drugs in Cabenuva 400-mg/600-mg Kit ER - Injection: Contains of vial of 400mg CAB in 2ml (200 mg/1ml) &amp; 600mg RILP in 2 ml (300 mg/1ml)

	Sample No	Label Claim (mg)	Result (mg)	% Label Claim	% Avg Assay	SD	% RSD
CAB	1	400	399.59	99.89	99.80	0.08	0.08
	2	400	399.15	99.78			
	3	400	398.89	99.72			
RILP	1	600	599.70	99.95	99.95	0.12	0.12
	2	600	598.99	99.83			
	3	600	600.44	100.07			

Table 9: Assay of Drugs in Cabenuva 600-mg/900-mg Kit ER - Injection: Contains of vial of 600mg CAB in 3ml (200 mg/1ml) &amp; 900mg RILP in 3ml (300 mg/1ml)

	Sample No	Label Claim (mg)	Result (mg)	% Label Claim	% Avg Assay	SD	% RSD
CAB	1	600	598.33	99.72	99.64	0.35	0.35
	2	600	599.74	99.95			
	3	600	595.57	99.26			
RILP	1	900	894.23	99.35	99.52	0.28	0.28
	2	900	898.72	99.85			
	3	900	894.23	99.36			

The method is helpful in the assay analysis for the drugs CAB & RILP, having % Assay results CAB 99.64-99.80% & RILP 99.52-99.95% as compared with the label claimed. The method has very accurate working response within very lower range of concentration of 2.5 to 15 $\mu\text{g/ml}$  for CAB and 3.75 to 22.5 $\mu\text{g/ml}$  for RILP in individual as well as in the dosage forms. This helps in the detection of the analyte drugs by using less amount of the working standards making the method very cost effective.

## CONCLUSION:

The developed analytical method is able to detect the drugs Cabotegravir CAB, Rilpivirine RILP in individual as well as in the samples vials and also in the combined dosage forms with the accuracy and precision parameters as per the ICH guideline. The Stability method i.e. forced degradation study helps to understand the different impurities, degraded products, generated in stress conditions and the method effectively detects the drug analytes pure peaks, without any

interference of other peaks. Also the analytical method is successfully validated as per the ICH guidelines and is useful for the assay and recovery study from the marketed formulations. The HPLC run time is 5mins which is beneficial and time saving for the quick analysis of the drugs in different individual dosage-forms as well as in the combinations.

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