https://doi.org/10.48047/AFJBS.6.15.2024.6162-6184

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Development and Validation of a Robust RP-HPLC Method for Simultaneous Estimation of Rilpivirine and Cabotegravir in Pharmaceutical Formulations

Rasapelly Ramesh Kumar[1]*, Bhoomika Vuppala[2], Parneetha Bangaru[2], Bodapati Uma Koushik[2], Nangunuri Prem Sai[2]

[1*]Department of Pharmaceutical Chemistry, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad.

[2]B Pharmacy Final Year, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad.

***Corresponding Author:** Dr Rasapelly Ramesh Kumar rameshkumarrasapelly@gmail.com

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.6162-6184

Abstract

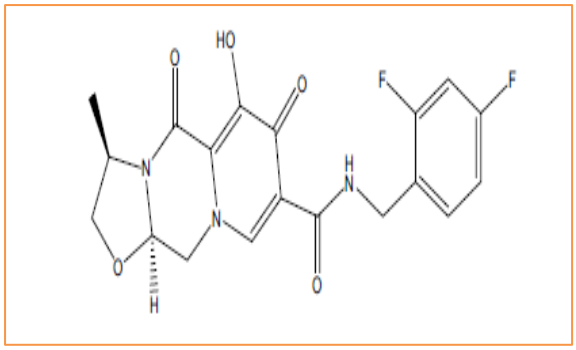
Novel RP-HPLC Method Development and Validation for Simultaneous Quantification of Rilpivirine and Cabotegravir in Pharmaceutical Formulations by Isocratic Separation using THERMO C18 Column. This method employs OPA-Methanol (50:50, pH 3.5) as the mobile phase at a flow rate of 1.0 mL/min, and UV detection at 219 nm. Retention times were 1.712 and 2.295 minutes for Rilpivirine and Cabotegravir, respectively. This method exhibited linearity in the concentration range of 50 µg/ml to 150 µg/ml for both drugs, with correlation coefficients of 0.9999. Limits of detection (LOD) and quantification (LOQ) were determined as 1.554 and 5.180 µg/ml for Rilpivirine, and 1.011 and 3.370 µg/ml for Cabotegravir. Excellent percentage recoveries of 100% for both analytes highlight the high accuracy of the proposed method. Specificity was confirmed through the correlation of retention times between standard and sample, ensuring interference-free determination of analytes in tablet dosage forms. The method underwent extensive validation following ICH guidelines, demonstrating robustness in terms of Linearity, Accuracy, Precision, Specificity, and Robustness.

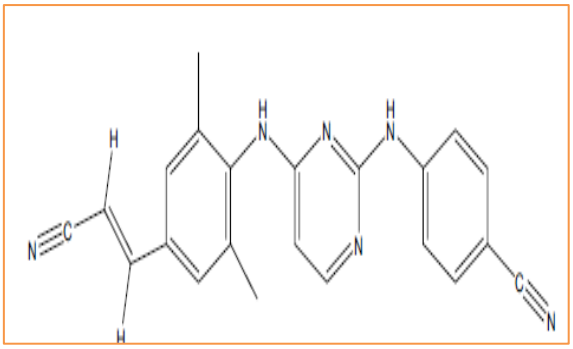
Keywords: Cabotegravir, High Performance Liquid Chromatography, Method Development, Rilpivirine, Validation

1. Introduction

A new, simple, efficient, quick, and exact reverse-phase high-performance liquid chromatography (RP-HPLC) approach was developed to estimate Cabotegravir and Rilpivirine in bulk and pharmaceutical dose forms. The newly established technique was later verified according to ICH recommendations in terms of linearity, accuracy, precision, the limit of detection, the limit of quantification, and robustness. Cabotegravir is a drug used to treat acquired immune deficiency syndrome.[1,2] It is available in tablet and intramuscular injection form[3,4] as well as an injectable combination with Rilpivirine sold under the brand name Cabenuva. The injectable forms are administered once a month or every two months. Cabotegravir combined with Rilpivirine has been demonstrated to treat human immune deficiency virus type 1 in adults. And, if the virus has not evolved resistance to the inhibitors, the combo injection will be used to treat people who do not have detectable human immune virus levels in their blood after receiving antiretroviral therapy[5,6] and integrate strand transfer inhibitors[7].

Before initiating injectable therapy, the tablets are used to determine how a person responds to the medicine. The two pharmaceuticals are the first antiretroviral medications to be accessible in an injectable form with an extended half-life. This means that instead of taking medications on a daily basis, people receive intramuscular injections once a month. Rilpivirine, commonly known as Edurant and Rekambys, is a Tibotec prescription drug used to treat the human immune virus and acquired immune deficiency syndrome[8]. It is a second-generation non-nucleoside reverse transcriptase inhibitor with fewer side effects, greater potency, and a longer half-life than previous non-nucleoside reverse transcriptase inhibitors such as efavirenz[9]. The injectable formulation's well-known adverse effects include injection site responses (in up to 84 percent of patients), such as pain and edema, cerebral ache[10,11] and fever[12,13] or feeling hot. Depressive problems, sleeplessness[14,15] and rashes are uncommon (less than 10%). The medications' less common adverse effects include depression[16], headaches, rashes, and sleeplessness. These adverse effects were seen when Rilpivirine was coupled with one or more additional anti-human immunological virus medications. Heart rhythm prolongation[17,18] has been seen at extremely high dosages of the medication, although it is not clinically significant at regular doses, the chemical structures of Carbotegravir and Rilpivirine represented in figure 1.





a. **b.**

Fig 1: Shows structures of a. Cabotegravir b. Rilpivirine.

2. Methods

2.1 Instrumentation

The study was carried out using a Water Alliance-e2695 chromatographic system outfitted with a quaternary pump and photodiode array detector-2996. Data was collected using the chromatographic program Empower 2.0.

2.2 Chemicals and reagents

Cabotegravir (HPLC grade), Rilpivirine (HPLC grade), and water (HPLC grade) were purchased from Merck (India) Ltd., Worli, Mumbai, India.

2.3 Chromatographic conditions

A reverse phase liquid chromatographic technique for estimating Cabotegravir and Rilpivirine in bulk pharmaceuticals and commercially available pharmaceutical dosage forms was developed and validated. Optimized chromatographic settings for maximum performance using Phenomenex Gemini (250mmx4.6mm) 5µm Particle size Column with guard filter. The separation was performed using a mobile phase comprising Methanol and Phosphate Buffer pH-4.2 in a 20:80v/v ratio, pumped at a flow rate of 1.0 mL/min, and detected at 246 nm. The technique was linear in the concentration ranges of 20-100 µg/mL and 40-120 µg/mL for Cabotegravir and Rilpivirine, with regression coefficients of 0.999 and 0.999, respectively[19].

2.4 Selection of wavelength

The absorption spectra of two pharmaceutical solutions were examined in the UV range 200-400 nm with a photodiode spectrophotometer. The spectra are shown in Figure 2. The spectra of Rilpivirine and Cabotegravir display unique λmax, which are 282nm and 265.4nm, respectively. The HPLC chromatographic procedure used two detection wavelengths of 262 nm on average[20]

2.5 Preparation of standard solution

100 mg of Cabotegravir and 50 mg of Rilpivirine working standards were precisely weighed and put to a 100 mL volumetric flask. Add 70 ml of mobile phase, sonicate for 20 minutes to dissolve the components, then dilute to the mark with diluent and mix thoroughly. Following that, 5 mL of the aforementioned solution was diluted to 50 mL with mobile phase [21].

2.6 Preparation of sample solution

Weighed 20 tablets and took one tablet's corresponding weight. Crush the 20 pills into powder, then transfer 10 tablets' equivalent weight of sample to a 100 ml volumetric flask with 70 ml of diluent and sonicate for 30 minutes. Make up to the volume with diluent. Dilute 5-50 ml with mobile phase and filter through a 0.45 µ nylon syringe filter.

3. Validation

3.1 System suitability

According to the test method, standard solutions were made and injected into an HPLC system, and the evaluated system suitability parameters were determined to be within the limits[22].

3.2 Specificity

The specificity defined as the method's capacity to quantify the analyse precisely and specifically in the presence of components in the sample matrix, was assessed by analysing chromatograms of drug-free and drug-added placebo formulations.

3.3 Linearity

The method's capacity to yield findings that are directly or indirectly proportional to the analyse concentration in samples within a specified range.\ Precision

The degree of agreement between individual test findings when the procedure is used to several samples of a homogenous sample. It measures the method's reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions)[23]

3.4 Accuracy

A technique was used to determine the degree to which the findings were near to the real value. It is a measurement of the method's accuracy.

3.5 Limit of detection and quantification

The detection and quantification limits for each analyse were obtained using a signal-to-noise concept, defined as the lowest concentration at which the signal-to-noise ratio is 3 or 2:1 and 10:1, respectively, with defined precision and accuracy under the provided experimental circumstances [24].

3.6 Stability

Standard and sample solutions were evaluated to 24 hour stability at room temperature and 2-8°C. The stability of these solutions was investigated by looking for changes in the area and retention duration of the peaks, which were then compared to the chromatogram pattern of the freshly created solution.

3.7Robustness

The method's robustness was tested by varying the experimental parameters such as flow rate and organic content. This was completed by the same analyst using the same instrument.

3.8 Ruggedness

The method's robustness was investigated utilizing a variety of analysers, equipment, wavelengths, and columns under identical experimental settings.

4. RESULTS AND DISCUSSION

4.1 Method validation

In this method, system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), forced degradation, and stability are validated for the selected Cabotegravir and Rilpivirine drugs.

4.2 System suitability

100 mg of Cabotegravir and 50 mg of Rilpivirine was prepared and injected into the HPLC system. Resolution was 1.712 and 2.295. The number of theoretical plate counts was 2638and 3014 respectively. Tailing factor for Cabotegravir and Rilpivirine was 1.47 and 1.35, respectively (Table 1).

4.3 Linearity

Linearity of the method was evaluated by preparing a standard solution containing 100 µg/ml of Cabotegravir and 50 µg/ml of Rilpivirine (100% of targeted level of the assay concentration). Sequential dilutions were performed to give solutions at 10, 25, 50, 100, and 150% of the target concentrations. These were injected and peak areas used to plot calibration curves against the concentration. The correlation coefficient values of these three analytes were 0.9998. The results are shown in Table 2 and 3 and Figure 1 and 2.

4.4 Limit of detection and quantification

Limit of detection and quantification minimum concentration level at which the analate can be reliably detected, quantified using the standard formulas (3.3 times σ/s for LOD and LOQ, respectively). LOD values for Cabotegravir and Rilpivirine were 1.554 µg/ml and 1.011µg/ml their s/n values are 3 and 4, respectively. LOQ values for 1.554 and 5.180 µg/ml for

Rilpivirine, and 1.011 and 3.370 µg/ml for Cabotegravir their s/n values are 23 and 26, respectively.

4.5 Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From this, six separate sample solutions were injected to obtain their areas. The calculate mean and percentage relative standard deviation (RSD) values. The present method was found to be precise as percentage RSD of <2%, and also, the percentage assay values were close to being 100%. The results are given in Table 4 and 5.

4.6 Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentrations levels (50, 100, and 150%). APIs with concentration of 50, 100, and 150 µg/ml of Cabotegravir; 25, 50, and 75 µg/ml of Rilpivirine were prepared. As per the test method, the test solution was injected three preparations each spike level and the assay was performed. The percentage recovery values were found to be in the range of 100.22–100.45% for Cabotegravir and 100.37–100.58% for Rilpivirine. RSD values were found to be <2%. The results are given in Table 6 and 7

4.7 Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, and column. The value of percentage of RSD was <2% and exhibits the ruggedness of the developed method.

Robustness of the method found to be percentage RSD should be <2%. Slightly variations were done in the optimized method parameters suchas flow rate (±0.2%) and organic content in mobile phase (±5%).

4.8 Stability

Stability of standard and sample solutions is studied initial to 24 h in stored at room temperature and 2–8 °C. They are injected at different time intervals. The difference between initial to 24 h percentage assay not more than 2.0%. There is no effect in storage conditions for Cabotegravir and Rilpivirine drugs. The results are shown in Table 8.

5. CONCLUSION

This method described the quantification of Cabotegravir and Rilpivirine in bulk and pharmaceutical formulation as per the ICH guidelines. The developed method was found to be accurate, precise, linear, and reliable. The advantage lies in the simplicity of sample preparation and the cost economic reagents were used. In addition, two compounds are eluted within 10 min. Moreover, also, same method is used for bio analytical plasma samples. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research

6. **Acknowledgments**

The authors are thankful to the management and principal of Marri Laxman Reddy Institute of Pharmacy, Dundigal, and Hyderabad for providing the facilities to complete this project work.

7. Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

8. References

1. De Cock KM, Jaffe HW, Curran JW (2012) The evolving epidemiology of HIV/AIDS. AIDS 26(10):1205–1213
2. Blankson JN (2010) Control of HIV-1 replication in elite suppressors. Discov Med 9(46):261–266
3. Sisson H (2015) Aspirating during the intramuscular injection procedure: a systematic literature review. J Clin Nurs 24(18):2368–2375
4. Şanlialp Zeyrek A, Takmak Ş, Kurban NK, Arslan S (2019) Systematic review and meta-analysis: physical-procedural interventions used to reduce pain during intramuscular injections in adults. J Adv Nurs 75(12):3346–3361
5. De Clercq E (1998) the role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. Antivir Res 38(3):153–179
6. Das K, Sarafianos SG, Clark AD Jr, Boyer PL, Hughes SH, Arnold E (2007) Crystal structures of clinically relevant Lys103Asn/Tyr181Cys double mutant HIV-1 reverse

transcriptase in complexes with ATP and nonnucleoside inhibitor HBY 097. J Mol Biol 365(1):77–89

7. Savarino A (2006) A historical sketch of the discovery and development of HIV-1 integrase inhibitors. Expert Opin Investig Drugs 15(12):1507–1522
8. Stellbrink HJ (2007) Antiviral drugs in the treatment of AIDS: what is in the pipeline? Eur J Med Res 12(9):483–495
9. Goebel F, Yakovlev A, Pozniak AL, Vinogradova E, Boogaerts G, Hoetelmans R, de Béthune MP, Peeters M, Woodfall B (2006) Short-term antiviral activity of TMC278—a novel NNRTI—in treatment-naive HIV- 1-infected subjects. AIDS. 20(13):1721–6
10. Cámara-Lemarroy CR, Rodriguez-Gutierrez R, Monreal-Robles R, Marfil- Rivera A (2016) Gastrointestinal disorders associated with migraine: a comprehensive review. World J Gastroenterol (Review) 22(36):8149–8160
11. Stovner L, Hagen K, Jensen R, Katsarava Z, RB Lipton, Scher A, Steiner T, Zwart JA (2007) The global burden of headache: a documentation of headache prevalence and disability worldwide. Cephalalgia Int J Headache 27(3):193–210
12. Dayal R, Agarwal D (2016) Fever in children and fever of unknown origin. Indian J Pediatr 83(1):38–43
13. White L, Ybarra M (2017) Neutropenic fever. Hematol Oncol Clin N Am 31(6):981–993
14. Wilson JF (2008) In the clinic. Insomnia. Ann Intern Med 14891:ITC13-1-ITC13-16
15. Drake CL, Roehrs T, Roth T (2003) Insomnia causes, consequences, and therapeutics: an overview. Depress Anxiety 18(4):163–176
16. Schuckit MA, Tipp JE, Bergman M, Reich W, Hesselbrock VM, Smith TL (1997) Comparison of induced and independent major depressive disorders in 2,945 alcoholics. Am J Psychiatry 154(7):948–957
17. Gillis AM (2013) Heart Rhythm Journal celebrates 10 years. Heart Rhythm 10(5):619
18. Poole JE (2020) Heart Rhythm O2: bringing up-to-date heart rhythm science and information to anyone, anywhere. Heart Rhythm 1(1):1–2
19. Nagasarapu M, Dananna G. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of paracetamol and flupirtine maleate in pure and pharmaceutical dosage forms. Journal of Young Pharmacists. 2015;7(2):81.
20. Manikanta Kumar A, Naga B, Prasad LN, Diwan PV. Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz in the

Pharmaceutical Dosage Form. Journal of Advanced Pharmacy Education & Research Oct-Dec. 2012;2(4).

21. Vasavi N, Patan A. Method development and validation for the simultaneous estimation of Atazanavir and Ritonavir in tablet dosage form by RP-HPLC. Indian Journal of Research in Pharmacy and Biotechnology. 2013 Nov 1;1(6):808.
22. Solanki VS, Bishnoi RS, Baghel R, Jain D. RP-HPLC method development and validation for simultaneous estimation of Cilnidipine, Atenolol and Chlorthalidone. Journal of Drug Delivery and Therapeutics. 2018 Dec 15;8(6-s):78-82.
23. Pradhan M, Alexander A. Development and Validation of a Robust RP-HPLC Method for Analysis of Calcipotriol in Pharmaceutical Dosage Form. Research Journal of Pharmacy and Technology. 2019;12(2):579-83.
24. Prasad PB, Satyanaryana K, Krishnamohan G. Development and Validation of a Method for Simultaneous Determination of Metformin and Saxagliptin in a Formulation by RP-HPLC. American journal of analytical chemistry. 2015;6(11):841

Table 1: System suitability data of Rilpivirine and Cabotegravir

Parameter	Rilpivirine	Cabotegravir	Acceptance Criteria
Retention time	1.712	2.295	+-10
Theoretical plates	2638	3014	>2500
Tailing factor	1.47	1.35	<2.00
% RSD	0.2	0.1	<2.00

Table : Specificity data for Rilpivirine and Cabotegravir

S .no	Sample name	Rilpivirine area	Rt	Cabotegravir Area	Rt
1	Standard	1435186	1.712	1862585	2.295
2	Sample	1428382	1.700	1832653	2.277
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

Table 6: Accuracy (%recovery) results of Rilpivirine

S.NO	Accuracy level	Sample Name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	250.00	148.500	149.22	100	100
		2	250.00	148.500	149.02	100	
		3	250.00	148.500	149.32	101	
2	100%	1	500.00	297.000	294.62	99	99
		2	500.00	297.000	293.17	99	
		3	500.00	297.000	295.51	99	
3	150%	1	750.00	445.500	444.98	100	100
		2	750.00	445.500	446.62	100	
		3	750.00	445.500	444.54	100	

Table 7: Accuracy (%recovery) results of Cabotegravir

S.NO	Accuracy level	Sample Name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	250.00	99.000	99.40	100	100
		2	250.00	99.000	99.34	100	
		3	250.00	99.000	99.25	100	
2	100%	1	500.00	198.000	196.98	99	99
		2	500.00	198.000	197.01	100	
		3	500.00	198.000	196.15	99	
3	150%	1	750.00	297.000	296.46	100	100
		2	750.00	297.000	295.35	99	
		3	750.00	297.000	296.69	100	

Table 4: Precision data for Rilpivirine

S.No	RT	Area	%Assay
injection1	1.700	1428382	99
injection2	1.701	1422412	98
injection3	1.700	1438031	99
injection4	1.699	1429260	99
injection5	1.692	1427648	98
injection6	1.692	1436470	99
Mean			99
Std. Dev.			0.40
% RSD			0.41

Table 5: Precision data for Cabotegravir

S.no	RT	Area	%Assay
injection1	2.277	1832653	98
injection 2	2.277	1834163	98
injection 3	2.276	1846466	99
injection 4	2.273	1839188	99
injection 5	2.263	1844786	99

injection 6	2.263	1835455	99
Mean			99
Std. Dev.			0.31
%RSD			0.31

Table 2: Linearity data for Rilpivirine

S.No	Conc (µg/ml)	RT	Area
1.	50	1.683	721551
2.	75	1.687	1067854
3.	100	1.692	1423176
4.	125	1.694	1781212
5.	150	1.698	2151536
Correlation coefficient (r^2)			0.9998

Table 3: Linearity data for Cabotegravir

S.No	Conc (µg/ml)	RT	Area
1.	50	2.255	925688
2.	75	2.254	1379137
3.	100	2.258	1830841
4.	125	2.257	2296025
5.	150	2.260	2762477
Correlation coefficient (r^2)			0.9999

Table : Robustness data for Rilpivirine

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	1.407	2791	1.47
Increased flow rate(1.2ml/min)	2.105	2970	1.46
Decreased temperature(20^0c)	1.537	2720	1.49
Increased temperature(30^0c)	1.870	2907	1.47
Decreased comp rate(5%)	1.537	2720	1.49
Increased comp rate(5%)	2.105	2970	1.46
Decreased pH(0.2)	1.700	2626	1.48
Increased pH(0.2)	1.701	2669	1.48
Decreased nm(2)	1.714	2638	1.47
Increased nm(2)	1.710	2584	1.48

Table : Robustness data for Cabotegravir

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	1.878	3100	1.35
Increased flow rate(1.2ml/min)	2.785	3359	1.36
Decreased temperature(20^0c)	2.055	3093	1.36
Increased temperature(30^0c)	2.482	5063	1.35
Decreased comp rate(5%)	2.055	3093	1.36
Increased comp rate(5%)	2.785	3359	1.36
Decrease pH(0.2)	2.277	2963	1.35

Increased pH(0.2)	2.277	3010	1.35
Decreased nm(2)	2.297	3024	1.35
Increased nm(2)	2.293	2977	1.36

Table : Rilpivirine and Cabotegravir degradation data

Condition	**Percent assay**		**Percent degradation**	
	Rilpivirine	**Cabotegravir**	**Rilpivirine**	**Cabotegravir**
0.1 N HCl	88.13	90.81	11.87	9.19
0.1N NaOH	92.68	93.55	7.32	6.45
30% H_2O_2	91.83	94.85	8.17	5.15
105^oC	87.11	88.83	12.89	11.17
Sunlight	93.21	90.82	6.79	9.18
Water	98.18	98.77	1.82	1.23

Table : Summary of validation data for Rilpivirine

S.NO	**PARAMETER**	**RESULT**	**ACCEPTENCE CRITERIA**
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 2638 1.47 1.712 0.2	 Not less than 2500 Not more than2 Not more than 2%
2	Specificity a) Blank interference b) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.41	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	50-150mcg/ml 0.9999	 Not less than 0.999
5	Accuracy (Mean % recovery) 50%	 100%	

	100% 150%	99% 100%	97.00 – 103.00%
6	Robustness a) Flow rate variation b) Temperature variation	All the system suitability parameters are within the limits.	

Table 22: Summary of validation data for Cabotegravir

S.NO	PAAMETER	RESULT	ACCEPTENCE CRITERIA
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 3014 1.35 2.295 0.1	 Not less than 2000 Not more than 2 Not more than 2
2	Specificity c) Blank interference d) Placebo interference	 Specific	 Specific
3	Method precision(%RSD)	0.31	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	50-150mcg/ml 0.9999	 Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	 100% 99% 100%	 97 - 103%
6	Robustness c) Flow rate variation d) Temperature variation	All the system suitability parameters are within the limits.	

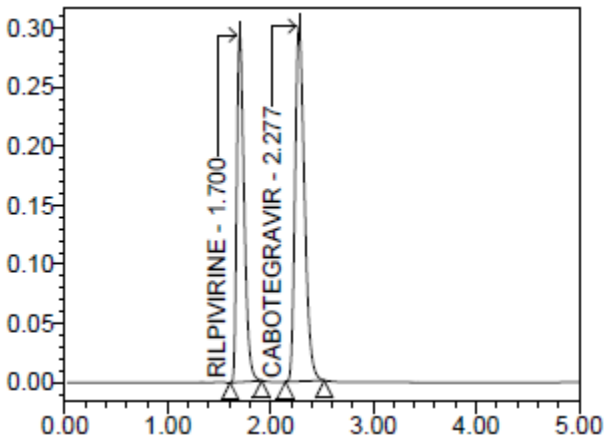


Fig 1: System suitability chromatography of Rilpivirine and Cabotegravir

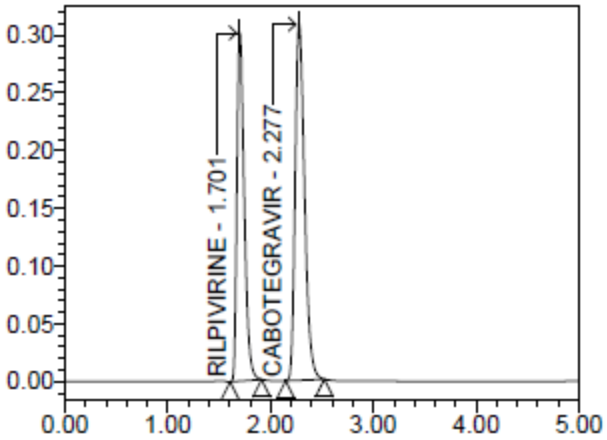


Fig 2: Chromatogram representing specificity of sample

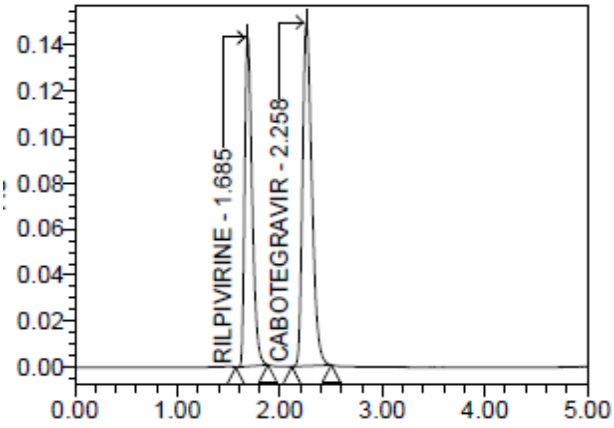


Fig 3: Typical chromatogram for Accuracy 50 %

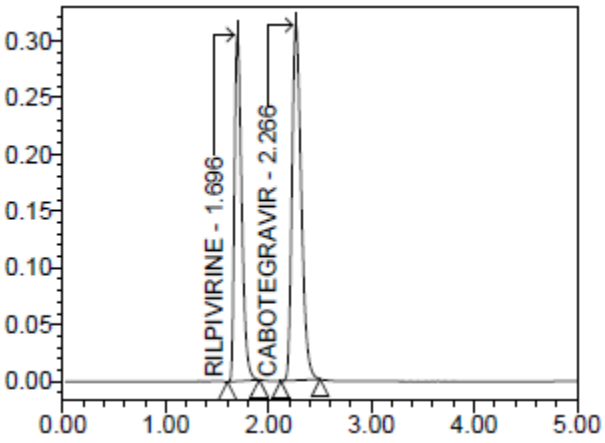


Fig 4: Typical chromatogram for Accuracy 100 %

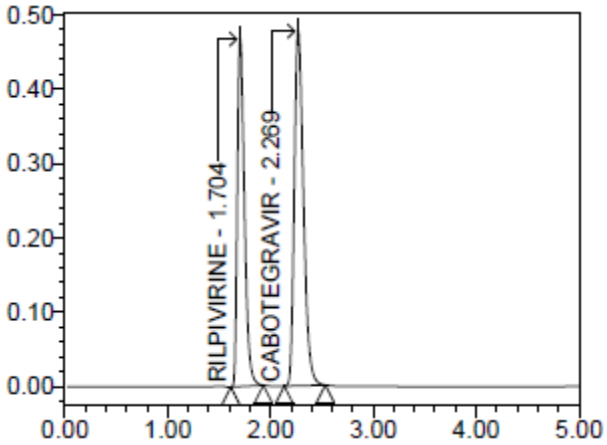


Fig 5: Typical chromatogram for Accuracy 150 %

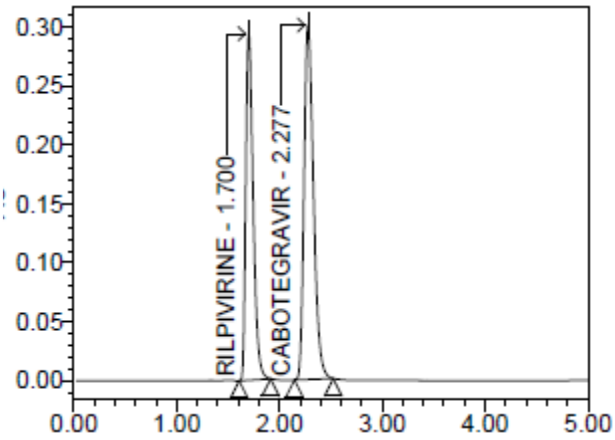


Fig 6: Chromatogram for precision injection 1

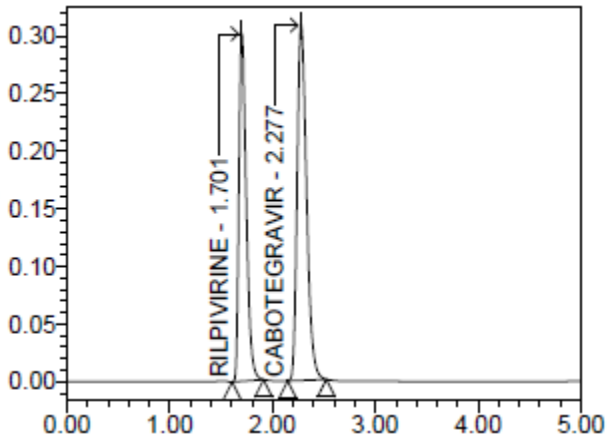


Fig 7: Chromatogram for precision injection 2

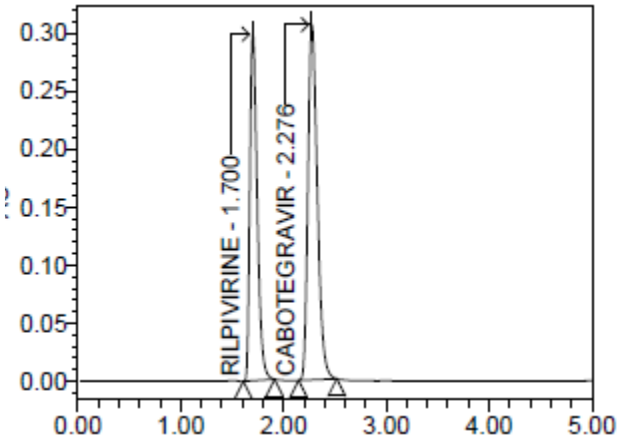


Fig 8: Chromatogram for precision injection 3

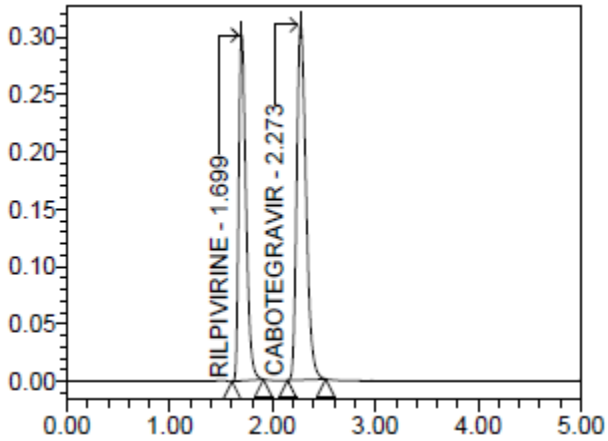


Fig 9: Chromatogram for precision injection 4

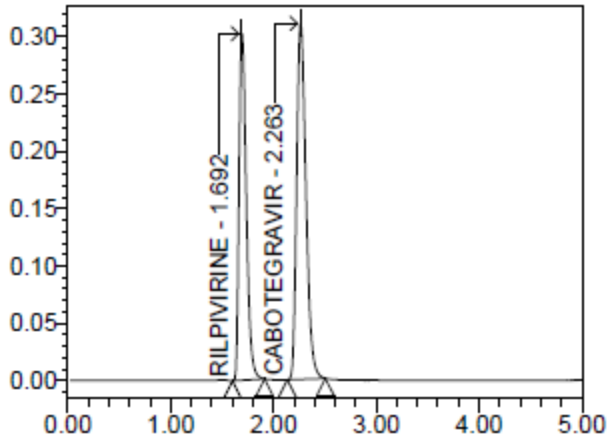


Fig 10: Chromatogram for precision injection 5

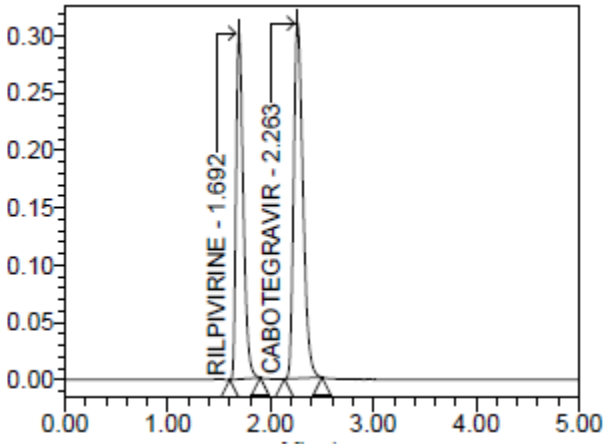


Fig 11: Chromatogram for precision injection 6

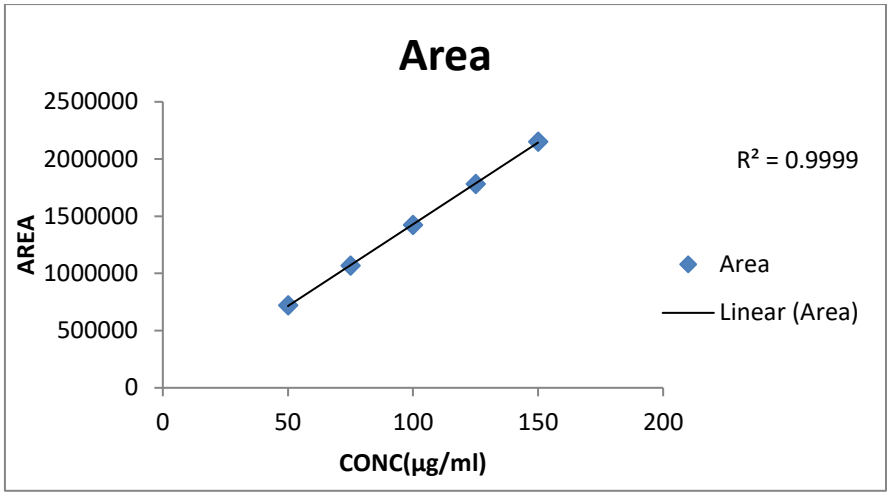


Fig 12: Linearity plot of Rilpivirine

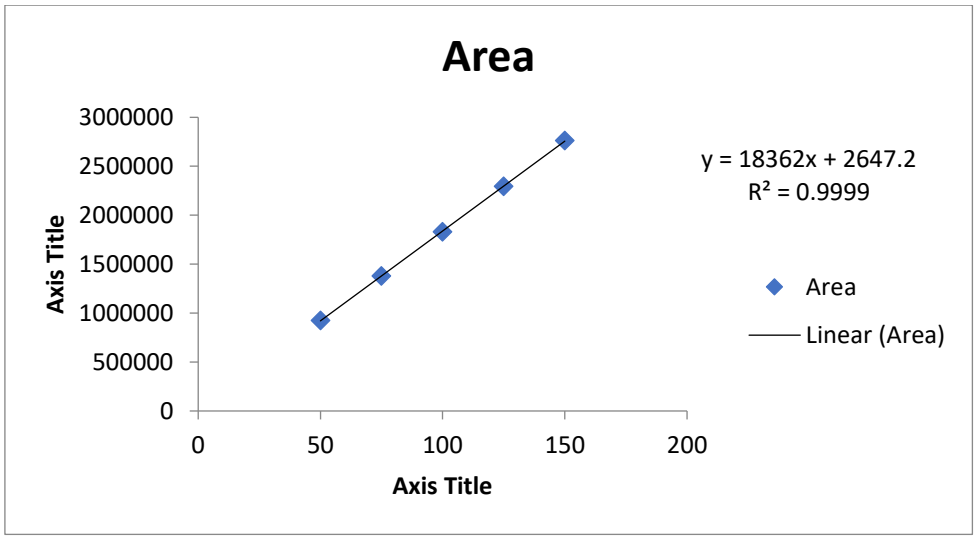


Fig 13: Linearity plot of Cabotegravir

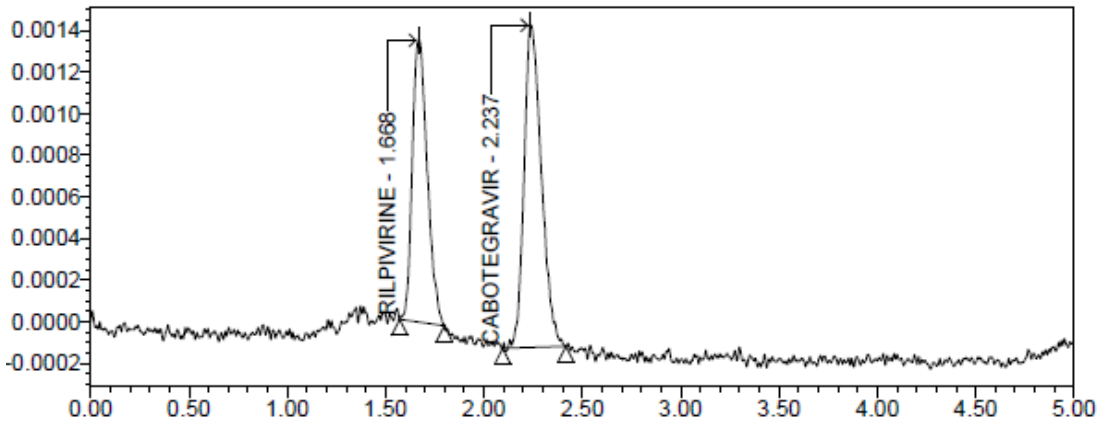


Fig 14: Chromatogram for LOD

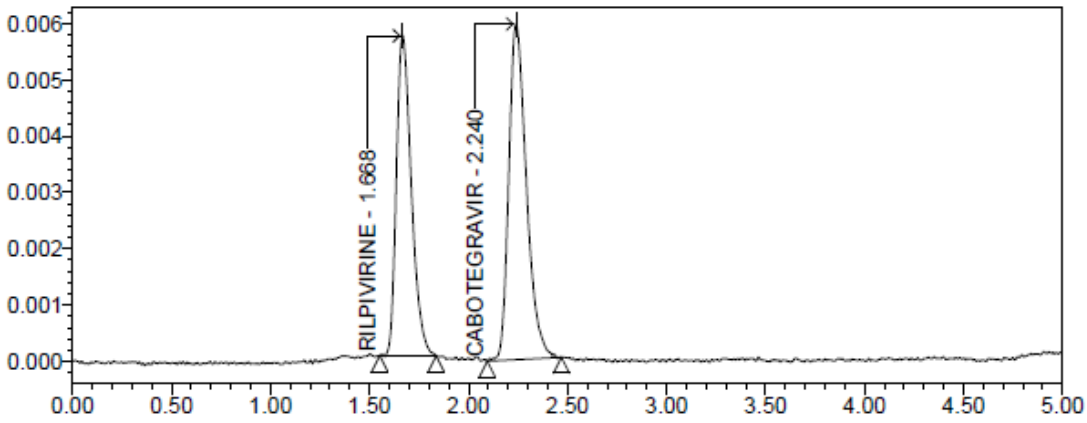


Fig 15: Chromatogram for LOQ

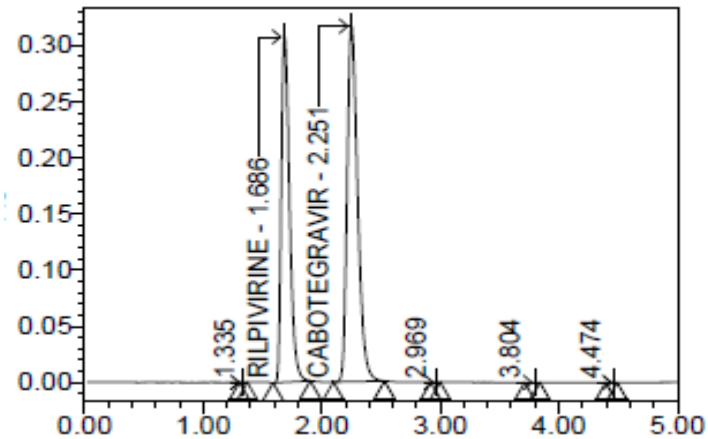


Figure 16: Acid degraded sample chromatogram

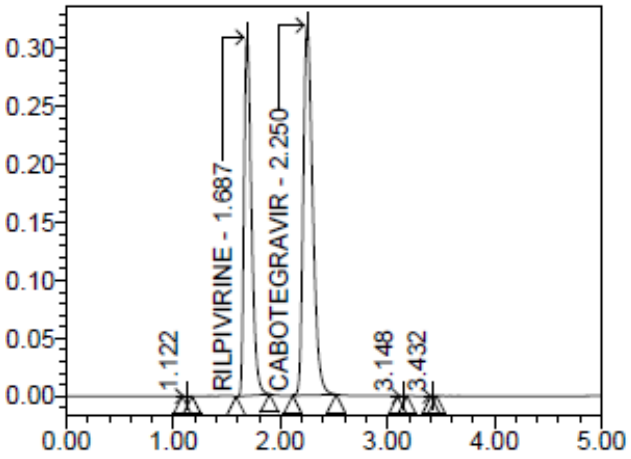


Fig 17: Base degraded sample chromatogram

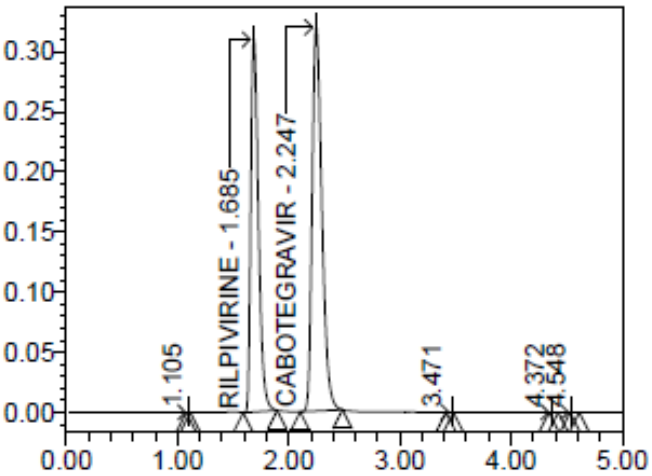


Fig 18: Oxidant degraded sample chromatogram

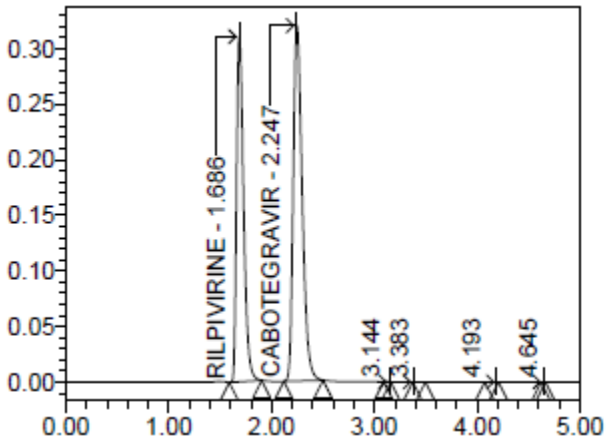


Fig 19: Thermal degraded sample chromatogram

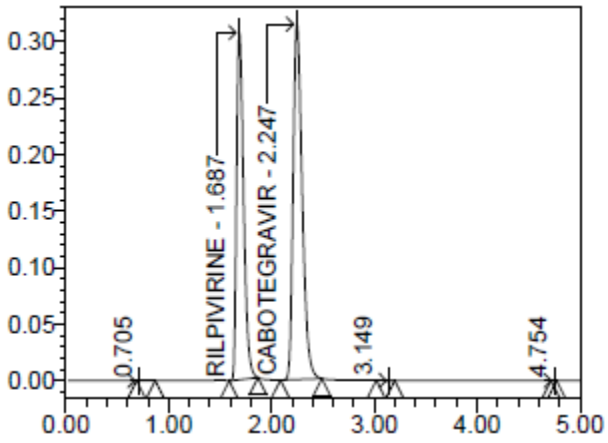


Fig 20: Photo degraded sample chromatogram

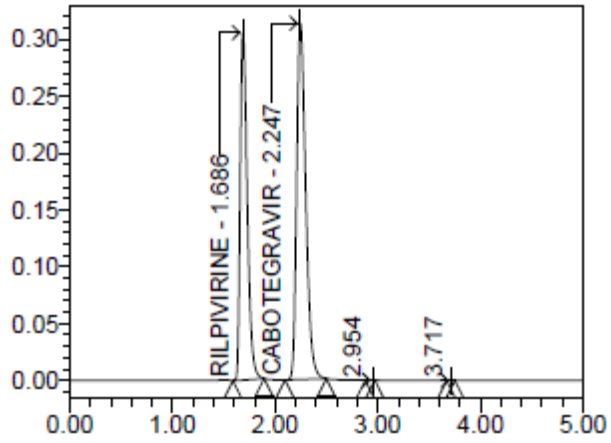


Fig 21: Water degraded sample chromatogram