ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CABOTEGRAVIR AND RILPIVIRINE IN BULK AND FORMULATION

Dasari Vasavi Devi*, D. Preethi Prathusha, G. Kiran Kumar, M. Divya sree, M. Lakshmi Priya, G.Azeez, S. Nelson Kumar

Department of Pharmaceutical Analysis, P. Rami reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

*Corresponding author E-mail: **dvas.reddy@gmail.com**

ARTICLE INFO

Key words:

Cabotegravir, Rilpivirine, RP-HPLC

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

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 BDS C8 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% ortho phosphoric acid: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.1% Ortho phosphoric acid buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 257 nm. Retention time of Cabotegravir and Rilpivirine were found to be 2.950 min and 3.518. %RSD of the Cabotegravir and Rilpivirine were and found to be 0.8 and 1.2 respectively. %Recovery was obtained as 99.19% and 98.90% for Cabotegravir and Rilpivirine respectively. LOD, LOQ values obtained from regression equations of Cabotegravir and Rilpivirine were 0.91, 2.77 and 1.05, 3.17 respectively. Regression equation of Cabotegravir is y = 12904x + 4984.8, and y = 11094x + 9528.6 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.

INTRODUCTION

Cabotegravir[1,2] is chemically (N-((2,4-Difluorophenyl) methyl)-6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11ahexahydro (1,3) oxazolo (3,2-a) pyrido(1,2-d) pyrazine-8- carboxamide. It is an integrase inhibitor with dolutegravir like carbamoyl pyridone structure. Cabotegravir is a medication used for the treatment of acquired immunodeficiency syndrome/HIV [3, 4]. It is available as tablets and as intramuscular injection. As an injectable blend with Rilpivirine under the brand name Cabenuva[5,6].

The structure of cabotegravir is shown in Figure 1. Rilpivirine is a non-nucleotide reverse transcriptase inhibitor used especially for treating HIV- 1 infections in treatment-naive patients. It is a derivative of diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Chemically Rilpivirine is 4-[[4- [(E)-2- cyanoethenyl] - 2, 6-dimethyl-anilino] pyrimidin- 2-yl] amino] benzonitrile)[7]. The structure of Rilpivirine was shown in Figure 2.

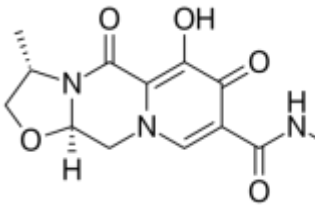

Figure 1: Structure of Cabotegravir

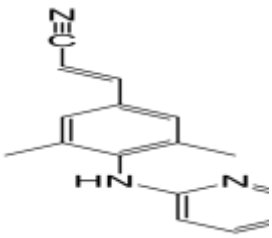

Figure 2: Structure of Rilpivirine

Literature review reveals that the HPLC determination of pure Rilpivirine has been reported for identification and quantification [8-20] and for Cabotegravir [21-24]. In the current study, a new, simple, sensitive and reliable RP-HPLC method for determination of Cabotegravir and Rilpivirine has been proposed.

MATERIALS AND METHODS:

Chemicals and Reagents:

Cabotegravir and Rilpivirine pure drugs (API), Combination Rilpivirine and Cabotegravir formulation (Cabenuva), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

HPLC Equipment 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 integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of Cabotegravir and Rilpivirine solutions.

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

Preparation of Standard stock solutions: To accurately weighed and transferred 150mg of Rilpivirine, and 100mg of Cabotegravir working Standards into a 50 ml clean dry volumetric flasks, 10ml of diluent was added, sonicated for 10 minutes and was made up to the final volume with diluents. (3000µg/ml Rilpivirine, and 2000µg/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. (300µg/ml Rilpivirine of and 200µg/ml of Cabotegravir)

Preparation of Sample stock solutions: 1ml of Rilpivirine and Cabotegravir injection sample was pipetted out 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. (3000µg/ml Rilpivirine, and 2000 µg/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. (300µg/ml of Rilpivirine and 200µg/ml of Cabotegravir)

Preparation of buffer:

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

VALIDATION:

The optimized method was validated as per ICH guidelines for the following parameters [25].

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Rilpivirine (300ppm) and Cabotegravir (200ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

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.

Precision: The standard working solution (300µg/ml Rilpivirine of and 200µg/ml of Cabotegravir) was injected for six times and measured the area for all six Injections in HPLC.

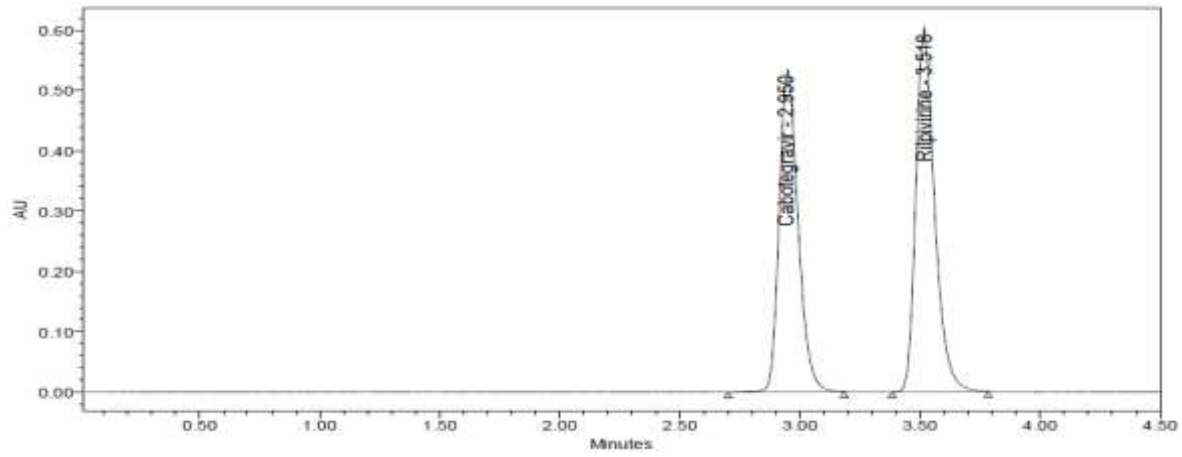

Figure 3: Optimised chromatogram

Table 1: System Suitability parameters for Cabotegravir and Rilpivirine

S. No.	Cabotegravir			Rilpivirine			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.904	6600	1.23	3.452	9301	1.31	3.80
2	2.905	6645	1.24	3.452	9391	1.31	3.8
3	2.905	6621	1.24	3.453	9496	1.33	3.7
4	2.908	6773	1.25	3.456	9901	1.33	3.8
5	2.928	6791	1.23	3.482	9485	1.29	3.8
6	2.950	6562	1.24	3.518	9437	1.31	3.9

Specificity:

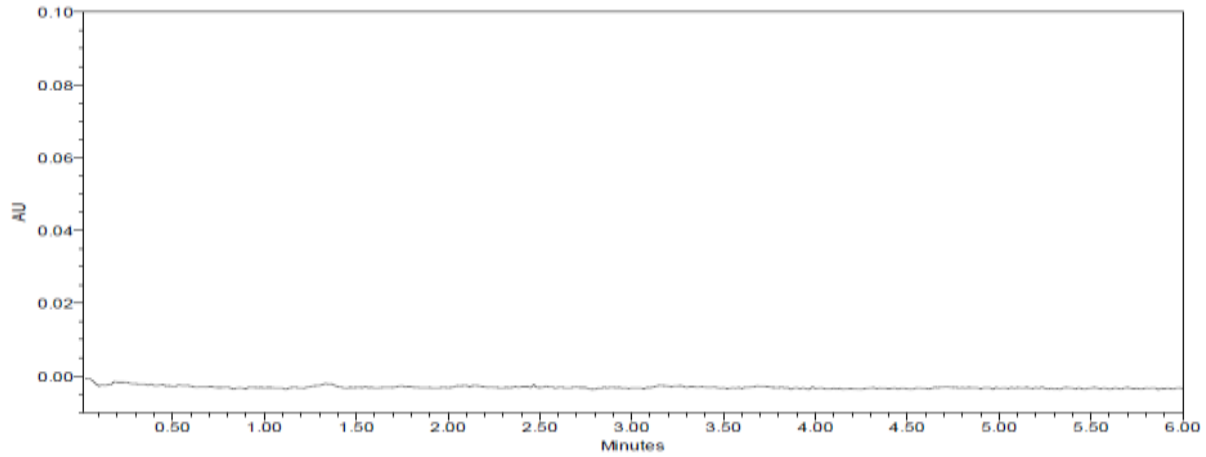

Figure 4: Chromatogram of blank

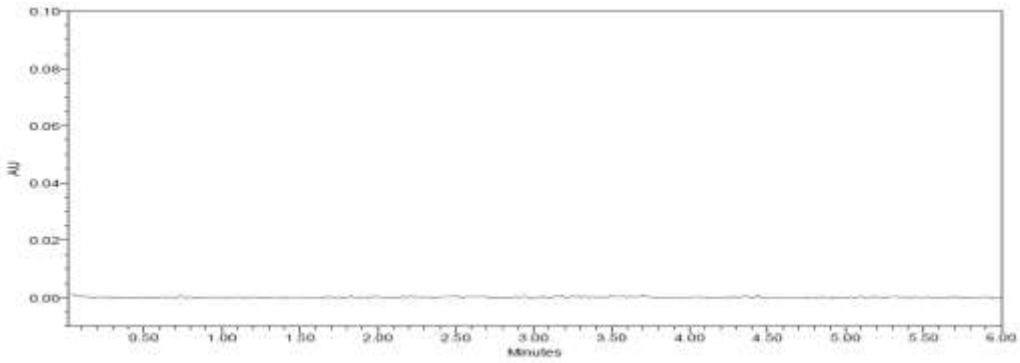

Figure 5: Chromatogram of placebo

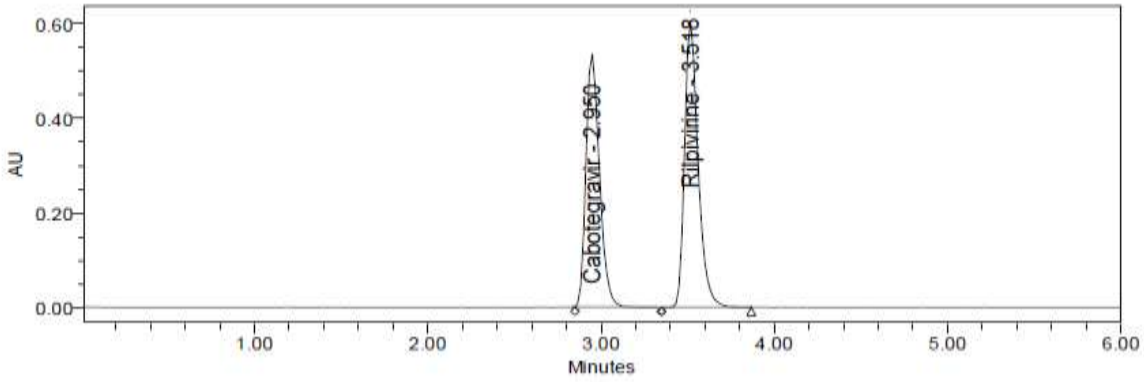

Figure 6: Typical Chromatogram of Cabotegravir and Rilpivirine

Table 2: Linearity table for Cabotegravir and Rilpivirine

Cabotegravir		Rilpivirine	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
50	612692	75	855164
100	1292004	150	1655467
150	1983700	225	2540136
200	2648572	300	3332570
250	3207198	375	4139110
300	3840069	450	5017599

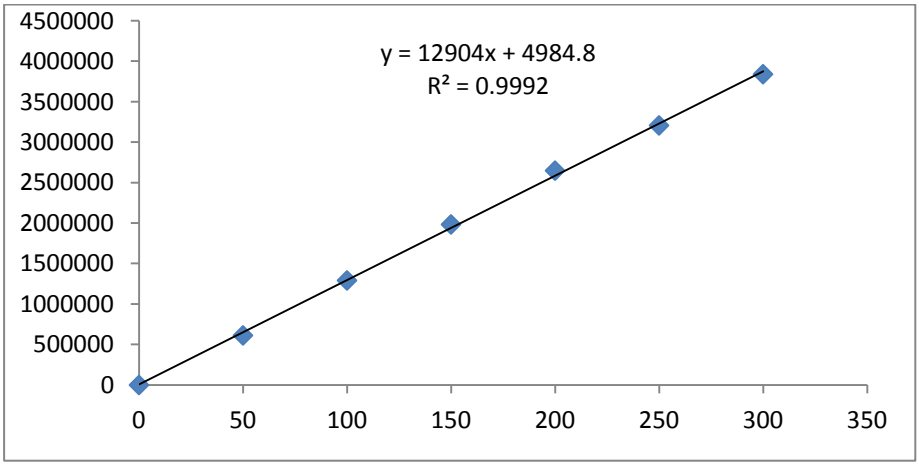

Figure 7: Calibration curve of Cabotegravir

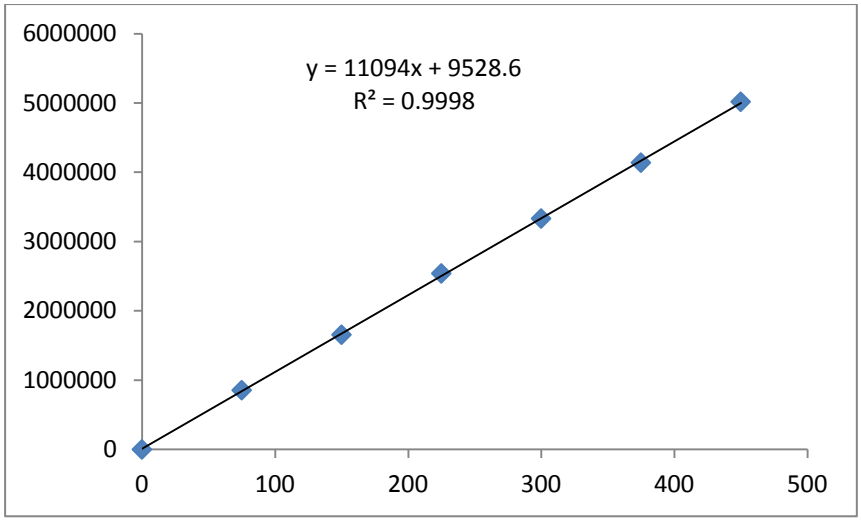

Figure 8: Calibration curve of Rilpivirine

Table 3: System precision table of Cabotegravir and Rilpivirine

S. No	Area of Cabotegravir	Area of Rilpivirine
1.	2631326	3251030
2.	2632272	3189535
3.	2641564	3229276
4.	2660079	3264240
5.	2633799	3179537
6.	2592280	3272005
Mean	2631887	3230937
S.D	22186.8	38879.9
%RSD	0.8	1.2

Table 4: Intermediate precision table of Cabotegravir and Rilpivirine

S. No	Area of Cabotegravir	Area of Rilpivirine
1.	2587758	3223769
2.	2612809	3223769
3.	2623880	3219505
4.	2647526	3210726
5.	2647526	3210437
6.	2610782	3250300
Mean	2621714	3223084
S.D	23190.6	14609.1
%RSD	0.9	0.5

Table 5: Accuracy table of Cabotegravir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	100	99.98	99.98	99.19%
	100	98.65	98.65	
	100	99.16	99.16	
100%	200	196.93	98.46	
	200	198.01	99.01	
	200	198.70	99.35	
150%	300	297.18	99.06	
	300	298.91	99.64	
	300	298.22	99.41	

Table 6: Accuracy table of Rilpivirine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	150	148.12	98.74	98.90%
	150	148.45	98.97	
	150	149.99	99.99	
100%	300	297.86	99.29	
	300	298.20	99.40	
	300	293.57	97.86	
150%	450	443.94	98.65	
	450	442.33	98.30	
	450	445.03	98.90	

Table 7: Sensitivity table of Cabotegravir and Rilpivirine

Molecule	LOD	LOQ
Cabotegravir	0.91	2.77
Rilpivirine	1.05	3.17

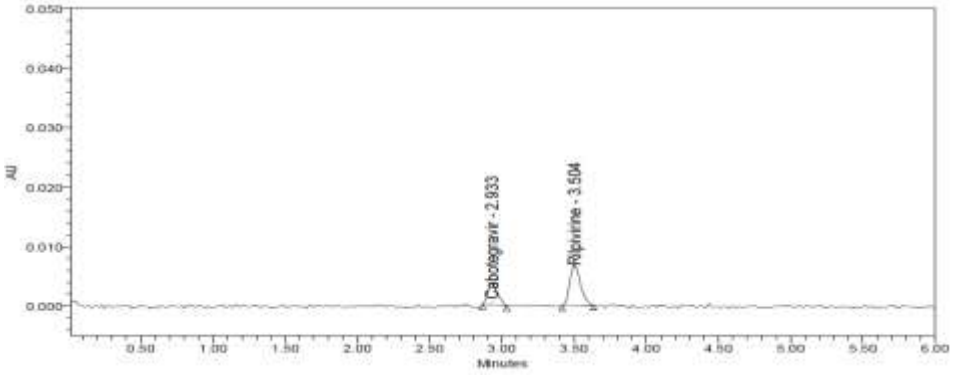

Figure 9: LOD Chromatogram of Standard

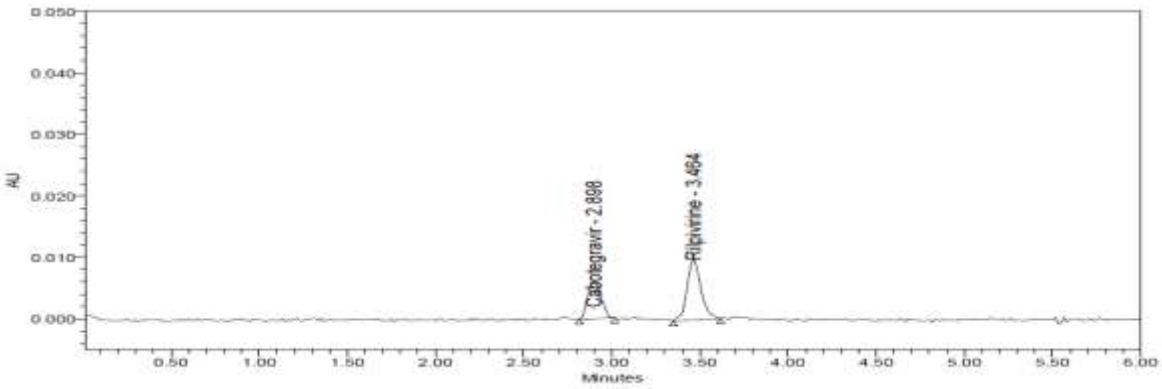

Figure 10: LOQ Chromatogram of Standard

Table 8: Robustness data for Cabotegravir and Rilpivirine

S.No	Condition	%RSD of Cabotegravir	%RSD of Rilpivirine
1	Flow rate (-) 0.9ml/min	0.6	0.3
2	Flow rate (+) 1.1ml/min	0.8	0.9
3	Mobile phase (-) 55B:45A	1.1	0.5
4	Mobile phase (+) 45B:55A	0.3	0.5
5	Temperature (-) 25°C	0.5	0.3
6	Temperature (+) 35°C	0.6	1.0

Table 9: Assay Data of Cabotegravir and Rilpivirine

S.No	Cabotegravir			Rilpivirine		
	Standard Area	Sample area	% Assay	Standard Area	Sample area	% Assay
1	2631326	2609346	98.95	3251030	3253887	100.51
2	2632272	2598605	98.54	3189535	3248542	100.34
3	2641564	2633491	99.86	3229276	3197201	98.76
4	2660079	2601683	98.65	3264240	3258275	100.64
5	2633799	2614810	99.15	3179537	3196741	98.74
6	2592280	2612297	99.06	3272005	3245148	100.24
Avg	2631887	2611705	99.03	3230937	3233299	99.87
Stdev	22186.8	12347.0	0.47	38879.9	28495.5	0.88
%RSD	0.8	0.5	0.5	1.2	0.9	0.88

Table 10: Degradation data of Cabotegravir and Rilpivirine

Type of degradation	Cabotegravir			Rilpivirine		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	2807620	93.97	6.03	3056655	94.42	5.58
Base	2858398	95.67	4.33	3077806	95.07	4.93
Peroxide	2895212	96.90	3.10	3104268	95.89	4.11
Thermal	2918531	97.68	2.32	3146655	97.20	2.80
UV	2954490	98.89	1.11	3193197	98.63	1.37
Water	2971160	99.44	0.56	3204268	98.98	1.02

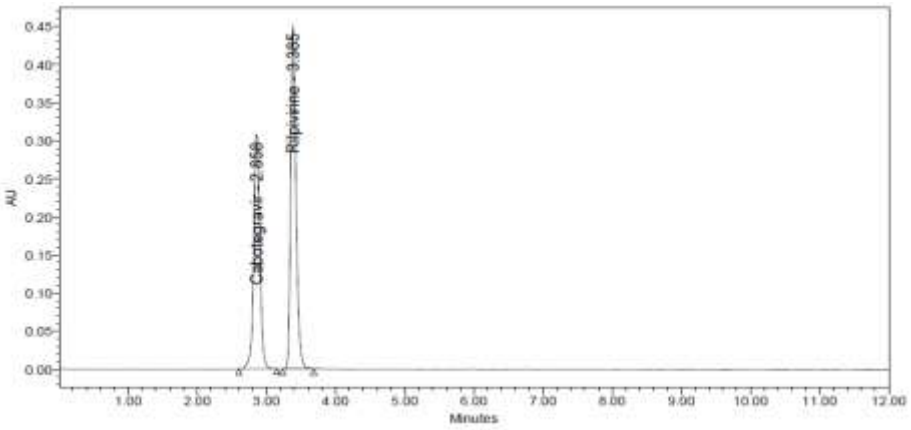

Figure 11: Acid degraded chromatogram

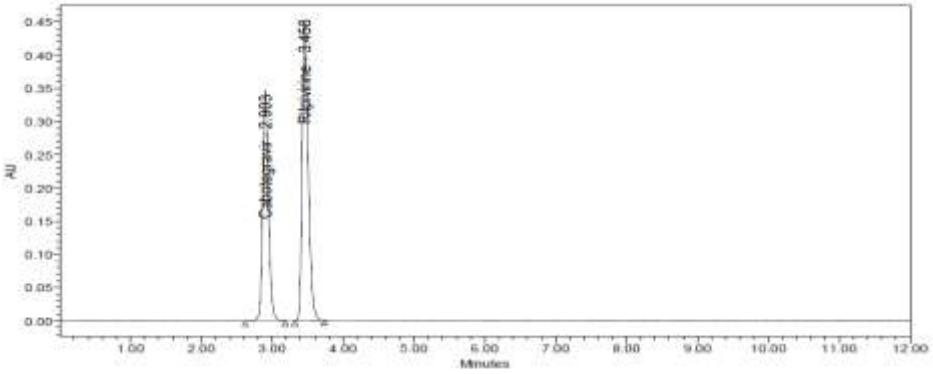

Figure 12: Base degraded chromatogram

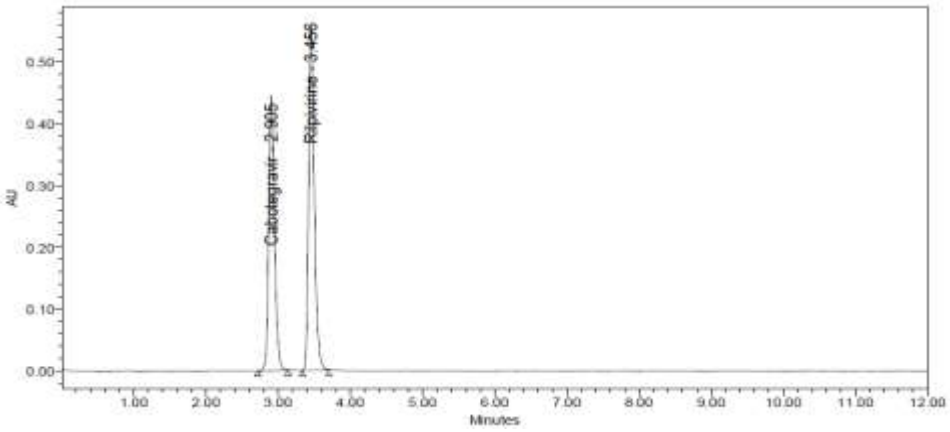

Figure 13: Peroxide degraded chromatogram

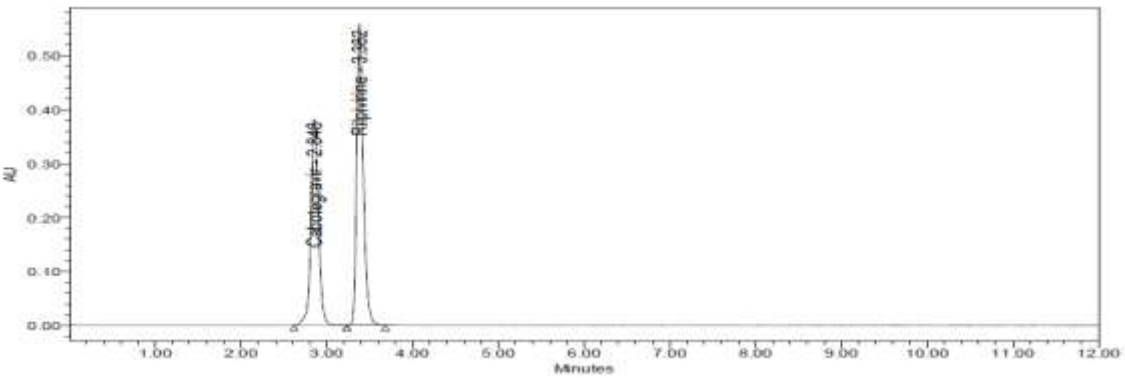

Figure 14: Thermal degraded chromatogram

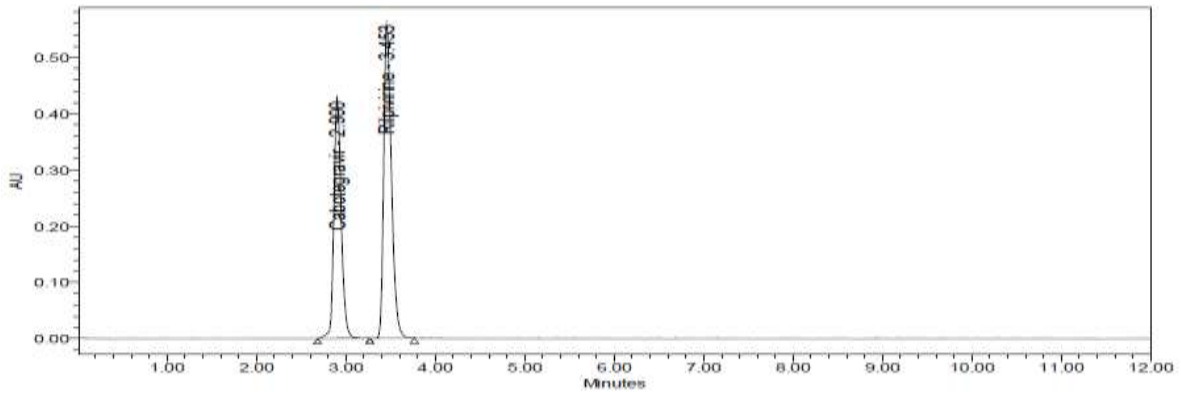

Figure 15: UV degraded chromatogram

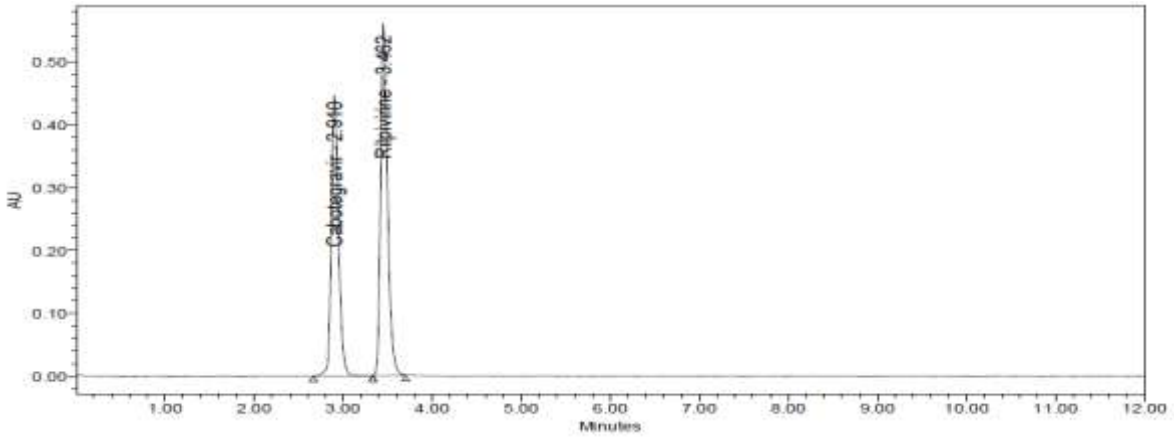

Figure 16: Water degraded chromatogram

Parameters		Cabotegravir	Rilpivirine	LIMIT
Linearity Range (µg/ml)		50-300µg/ml	75-450 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		12904	11094	
Intercept(c)		4984.8	9528.6	
Regression equation (Y=mx+c)		y = 12904x + 4984.8	y = 11094x + 9528.6.	
Assay (% mean assay)		99.03%	99.87%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.8	1.2	NMT 2.0%
Method precision- %RSD		0.5	0.9	NMT 2.0%
Accuracy %recovery		99.19%	99.90%	98-102%
LOD		0.91	1.05	NMT 3
LOQ		2.77	3.17	NMT 10
Robustness	**FM**	0.6	0.3	%RSD NMT 2.0
	FP	0.8	0.9	
	MM	1.1	0.5	
	MP	0.3	0.5	
	TM	0.5	0.3	
	TP	0.6	1.0	

Intermediate Precision: To evaluate the intermediate precision of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for six times and measured the area for all six injections in HPLC.

Linearity:

Preparation of Standard stock solutions: To accurately weighed and transferred 150mg of Rilpivirine, and 100mg of Cabotegravir working Standards into a 50 ml clean dry volumetric flasks, 10ml of diluent was added, sonicated for 10 minutes and made up to the final volume with diluents. (3000µg/ml Rilpivirine, and 2000µg/ml of Cabotegravir)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Rilpivirine and 50µg/ml of Cabotegravir)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (150µg/ml of Rilpivirine and 100µg/ml of Cabotegravir)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (225µg/ml of Rilpivirine and 150µg/ml of Cabotegravir)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (300µg/ml of Rilpivirine and 200µg/ml of Cabotegravir)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (375µg/ml of Rilpivirine and 250µg/ml of Cabotegravir)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (450µg/ml of Rilpivirine and 300µg/ml of Cabotegravir)

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. Acceptance Criteria: Correlation coefficient should be not less than 0.999.

Accuracy:

Preparation of Standard stock solutions: To accurately weighed and transferred 150mg of Rilpivirine, and 100mg of Cabotegravir working Standards into a 50 ml clean dry volumetric flasks, 10ml of diluent was added, sonicated for 10 minutes and made up to the final volume with diluents. (3000µg/ml Rilpivirine, and 2000µg/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 pipette 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 pipette out, and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipette 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 pipette out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Rilpivirine, Cabotegravir, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Robustness: A small deliberate change in method like Flow rate, mobile phase ratio,

and temperature are made but there were no recognized change in the result and was within range as per ICH Guide lines. Robustness conditions like Flow minus (1ml/min), Flow plus (1.2ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Degradation studies:

Acid Degradation Studies:

To 1 ml of stock solution Rilpivirine and Cabotegravir, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C.

Alkali Degradation Studies: To 1 ml of stock solution Rilpivirine and Cabotegravir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C.

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 60°C.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 1 hr to study dry heat degradation.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 3000µg/ml & 2000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60ºC. For HPLC study, the resultant solutions were diluted to obtain 300µg/ml & 200µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

Method Development

The chromatographic method development for the simultaneous estimation of Cabotegravir and Rilpivirine were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Cabotegravir and Rilpivirine in API and pharmaceutical dosage form by RP-HPLC method.

Optimized Chromatographic conditions:

Mobile phase	50% OPA (0.1%): 50% Acetonitrile
Flow rate	1 ml/min
Column	BDS C8 (4.6 x 150mm, 5µm)
Detector wave length	257 nm
Column temperature	30°C
Injection volume	10µL
Run time	6 min

Both peaks were eluted with good resolution and USP Plate count, USP tailing values were within limit based on ICH Guidelines.

Cabotegravir and Rilpivirine were eluted at 2.950 min and 3.518 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

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. All the system suitable parameters were passed and were within the limits. Retention times of Cabotegravir and Rilpivirine were 2.950min and 3.518 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. Six linear concentrations of Cabotegravir (50-300µg/ml) and Rilpivirine (75-450µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Cabotegravir was y = 12904x + 4984.8 and of Rilpivirine was y = 11094x + 9528.6.

Correlation coefficient obtained was 0.999 for the two drugs.

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 0.8% and 1.2% respectively for Cabotegravir and Rilpivirine. As the limit of Precision was less than "2" the system precision was passed in this method. Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.9% and 0.5% respectively for Cabotegravir and Rilpivirine. As the limit of Precision was less than "2" the system precision was passed in this method.

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 99.19% and 98.90% for Cabotegravir and Rilpivirine respectively.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay: Cabenuva, bearing the label claim Cabotegravir 600mg, Rilpivirine 400mg. Assay was performed with the above formulation. Average % Assay for Cabotegravir and Rilpivirine obtained was 99.03% and 99.87% respectively.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Cabotegravir and Rilpivirine in pharmaceutical dosage form. Retention time of Cabotegravir and Rilpivirine were found to be 2.950 min and 3.518. %RSD of the Cabotegravir and Rilpivirine were and found to be 0.8 and 0.5 respectively. %Recovery was obtained as 99.19% and 98.90% for Cabotegravir and Rilpivirine respectively. LOD, LOQ values obtained from regression equations of Cabotegravir and Rilpivirine were 0.91, 2.77 and 1.05, 3.17 respectively. Regression equation of Cabotegravir is y = 12904x + 4984.8, and y = 11094x + 9528.6 is 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.

REFERENCES

1. De Cock KM, Jaffe HW, Curran JW. The evolving epidemiology of HIV/AIDS. AIDS. 2012;26(10):1205–1213. Available from: https://doi.org/10.1097/qad.0b013e328354622a.
2. Blankson JN. Control of HIV-1 replication in elite suppressors. Discov Med. 2010;9(46):261 266. Available from: https://pubmed.ncbi.nlm.nih.gov/20350494/.
3. De Clercq E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. Antiviral Res. 1998; 38(3):153–179. Available from: https://doi.org/10.1016/s0166-3542(98)00025-4.
4. Das K, Sarafianos SG, Clark AD, Boyer PL, Hughes SH, Arnold E. Crystal Structures of Clinically Relevant Lys103Asn/Tyr181Cys Double Mutant HIV-1 Reverse Transcriptase in Complexes with ATP and Non-nucleoside Inhibitor HBY 097. Journal of Molecular Biology. 2007;365(1):77–89.

Available from: https://doi.org/10.1016/j.jmb.2006.08.097.
5. Sisson H. Aspirating during the intramuscular injection procedure: a systematic literature review. Journal of Clinical Nursing. 2015;24(17-18):2368–2375. Available from: https://doi.org/10.1111/jocn.12824.
6. Zeyrek Ş, Takmak A, Kurban Ş, Arslan NK, S. Systematic review and meta- analysis: Physical-procedural interventions used to reduce pain during intramuscular injections in adults. J Adv Nurs. 2019;75(12):3346–3341. Available from: https://doi.org/10.1111/jan.14183.
7. Goebel F, Yakovlev A, Pozniak AL, Vinogradova E, Boogaerts G, Hoetelmans R, et al. Short-term antiviral activity of TMC278–a novel NNRTI–in treatment-naive HIV-1-infected subjects. AIDS. 2006;20(13):1721–1726. Available from: https://doi.org/10.1097/01.aids.0000242818.65215.bd.
8. Venkatesan S, Kannappan N, Mannemala SS. Stability-Indicating HPLC Method for the Simultaneous Determination of HIV Tablet Containing Emtricitabine, Tenofovir Disoproxil Fumarate, and Rilpivirine Hydrochloride in Pharmaceutical Dosage Forms. Int Sch Res Notices. 2014;2014(849149). Available from: https://doi.org/10.1155/2014/849149.
9. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, Back D. Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLCMS/ MS) assay for the simultaneous determination of existing and new antiretroviral compounds .J Chromatogr B Analyt Technol Biomed Life Sci 2010 ; 878(19): 1455-65.
10. Aouri M, Calmy A, Hirschel B, Telenti A, Buclin T, Cavassini M, Rauch A, Decosterd LA. A validated assay by liquid chromatography-tandem mass spectrometry for the simultaneous quantification of elvitegravir and rilpivirine in HIV positive patients. J Mass Spectrom 2013; 48(5): 616-25.
11. Burugula L, Pilli NR, Makula A, Lodagala DS, Kandhagatla R. Liquid chromatography tandem mass spectrometric assay for the non-nucleoside reverse transcriptase inhibitor rilpivirine in human plasma. Biomed Chromatogr 2013; 27(2): 172-8.
12. Mohanareddy C, Hussain Reddy K, Narayanareddy P, Venkataramana M. Degradation pathway for rilpivirine hydrochloride by validated stability indicating UP-LC method. Int J Pharmacol clin Toxicol 2012; 1(1) :1-8.
13. Bhavar G. B, Pekamwar S. S, Aher K. B, Chaudhari S. R. Development and validation of UV Spectrophotometric method for estimation of rilpivirine hydrochloride in bulk and pharmaceutical formulations. Am. J. PharmTech Res. 2013; 3(1):450–458.
14. Somsubhra G, Sowjanya B, Laxmi P.V, Vidyadhar S, David B, Subhadip R. Method development and validation of rilpivirine in bulk and tablet doses form by RP-HPLC method. Res J Pharm Technol 2013; 6(3):240–243.
15. Sudha T, Shanmugasundram P. Reverse phase high performance and HPTLC methods for the determination of rilpivirine bulk and in tablet dosage form. World J Pharm Res 2012; 1(4):1183–196.
16. Kavitha K. Y, Geetha G, Hariprasad R, Venkatnarayanan R, Kaviarasu M. Development and validation of RP-UPLC analytical method for

simultaneous estimation of the emtricitabine, tenofovirdisoproxilfumerate and rilpivirine and its pharmaceutical dosageform. Int J Pharm Res 2013; 4(1):150–155.

17. Venkatesan S, Kannappan N. Simultaneous Spectrophotometric Method for Determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Three-Component Tablet Formulation Containing Rilpivirine Hydrochloride. Int Sch Res Notices. 2014:541727.
18. Date AA, Shibata A, Bruck P, Destache CJ. Development and validation of a simple and isocratic reversed-phase HPLC method for the determination of rilpivirine from tablets, nanoparticles and HeLa cell lysates. Biomedical chromatography : BMC 2015; 29(5):709- 715.
19. B M S Kumar, B. Rajkamal, B. Chandramowli. Development and Validation of Rilpivirine in Pharmaceutical Formulation by RP-HPLC. Am. J. PharmTech Res. 2019; 9(03); 345-353.
20. Dasari Vasavi devi, Dugasani Swarnalatha, Gopireddy Venkata Subbareddy. Optimization of RP-HPLC method for simultaneous estimation of Dolutegravir and Rilpivirine in binary mixture by using design of experiments. J. Global Trends Pharm Sci, 2019; 10(2): 6298 – 6310.
21. Lidija Kovac, Zdenko Casar, Tina Trdan Lusin, and Robert Roškar. Development of an Analytical Method for Determination of Related Substances and Degradation Products of Cabotegravir Using Analytical Quality by Design Principles. ACS Omega 2022, 7, 8896−8905.
22. Ramöller, I. K., Abbate, M. T. A., Vora, L. K., Hutton, A. R. J., Peng, K., Volpe- Zanutto, F., Tekko, I. A., Moffatt, K., Paredes, A. J., McCarthy, H. O., & Donnelly, R. F. (2022). HPLC-MS method for simultaneous quantification of the antiretroviral agents rilpivirine and cabotegravir in rat plasma and tissues. Journal of Pharmaceutical and Biomedical Analysis, 213, 114698.
23. Anuradha Vejendla, Subrahmanyam Talari, Raju Moturu, S. N. Murthy Boddapati and A. Emmanuel Kola. Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR. Future Journal of Pharmaceutical Sciences, 7(1), 226.
24. Courlet P, Saldanha SA, Cavassini M, Marzolini C, Choong E, Csajka C, et al. Development and validation of a multiplex UHPLC-MS/MS assay with stable isotopic internal standards for the monitoring of the plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine, and rilpivirine in people living with HI. Journal of Mass Spectrometry. 2020;55(6):e4506. Available from: https: //doi.org/10.1002/jms.4506.
25. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1), Geneva, November (2005).