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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF CABOTEGRAVIR AND RILPIVIRINE USING RP-HPLC

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Type I HIV can be treated by the combination of these drugs. An accurate, precise, and novel RP-HPLC technique was developed and validated for concurrent assessment of cabotegravir and rilpivirine in bulk and medicinal formulation. The separation was done on Waters 2695 HPLC system that comprised of PDA detector. Mobile phase was in the ratio of (70:30 v\v) acetonitrile and 0.1N potassium di hydrogen phosphate. Flow rate of 1 ml\min was employed. The detector wavelength was at 257nm.The run time was 5 minutes. The R^2 value for cabotegravir was observed to be y=7596.9x+1542.1 and for rilpivirine it was y=7517.8x+5409. LOD values for cabotegravir and rilpivirine were observed to be 0.25 µg/ml and 1.79 µg/ml respectively. LOQ values for cabotegravir and rilpivirine were found to be 0.77 µg/ml and 5.44 µg/ml respectively. The suggested approach was demonstrated to be exact, precise, and ideal for use in quality control laboratories for the quantifiable examination of dosage forms, both singular and mixed

Keywords*: Cabotegravir, Rilpivirine, C18 column, Validation, Method development*

INTRODUCTION

Antiretroviral medication cabotegravir is a structural counterpart of dolutegravir. Carbotegravir interacts with the HIV integrase active site to prevent the viral genome from transferring strands into the host genome and to halt virus replication. Due to the daily oral tablet administration and the monthly intramuscular suspension administration, the medication has a protracted period of action. Rilpivirine belongs to the class of compounds known as diary pyrimidines, similar to the pyrimidine nucleotides in DNA. A non-nucleoside reverse transcriptase inhibitor (NNRTI) called rilpivirine is used to treat HIV-1 infections in people who have never had therapy[1]. **Fig. 1 and 2** represent the chemical structures of cabotegravir and rilpivirine, respectively. According to literature survey, it was found that RP-HPLC[1-7], LC-MS[8], UPLC[9] methods were the works performed till date for both drugs in combination. Cabotegravir and rilpivirine are important because they are used to treat HIV and are the first complete long-acting injectable regimen for HIV-1 infection. Since the existing methods for these drugs were not economical and green methods, cabotegravir and rilpivirine were selected to developd a much economical and precise method. The study's main goal was to develop a simple, accurate RP-HPLC method for measuring rilpivirine and cabotegravir in pharmaceutical and pure dose forms.

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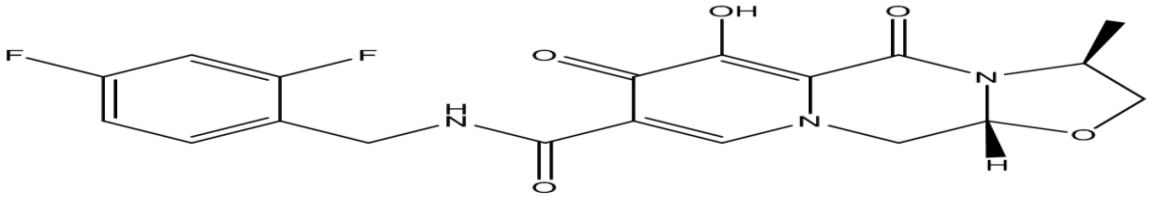

Fig. 1: Structure of cabotegravir.

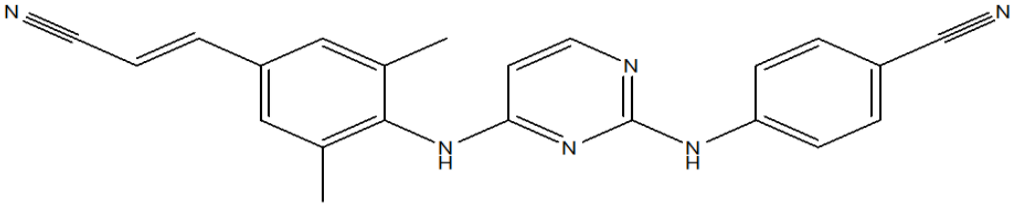

Fig. 2: Structure of rilpivirine

MATERIALS AND METHODS

Chemicals and reagents

A complimentary sample of cabotegravir and rilpivirine was given by Spectrum Pharma Research Solutions (Hyderabad). Rankem Laboratories Pvt. Ltd. provided the potassium dihydrogen ortho phosphate (KH_2PO_4) buffer, acetonitrile, ortho-phosphoric acid, and methanol. In the lab, Millipore Milli Q water was made.

Instruments and equipment

The Waters 2695 HPLC system was utilized, which was outfitted with photodiode array detector and quaternary pumps. A pH meter was utilized to assess the solutions' pH (BVK enterprises, India). On an analytical balance (Denver), all measurements were completed.

Preparation of stock solutions

Rilpivirine of weight 37.5 mg and cabotegravir of weight 25 mg standards were accurately weighed and placed into a fifty millilitres clean dry volumetric flask. 10 milliliters of diluent was added. Then, this solution was sonicated for ten minutes. Diluents were used to make up the final volume. A solution of 1000 µg/ml was obtained.

Preparation of sample working solution

A volumetric flask of capacity 10 ml was filled with diluent after pipetting out and adding 1 milliliter of stock solution. A sample working solution of 100 µg/ml was obtained

Preparation of diluents

Diluent was prepared by taking acetonitrile and water in the ratio 50:50 v/v.

Preparation of sample stock solutions

One milliliter of the cabotegravir and rilpivirine injection sample was pipetted into a volumetric flask of 100 ml volume along with 50 ml of diluents. The mixture was then subjected to twenty-five minutes of sonication. Finally, diluent was added to the volume (1000 µg/ml) and filters were used to remove impurities.

Preparation of sample working solutions

After being filtered, 0.5 milliliter of the sample stock solution was transferred to a volumetric flask of 10 ml volume and diluted with diluents.

Chromatographic conditions

Using a Kromasil C18 column, the RP-HPLC technique for rilpivirine and cabotegravir was developed and validated. 0.1N KH_2PO_4: acetonitrile was used in the mobile phase at a flow rate of 1 ml/min in a 70:30 v/v ratio. The injector volume for the sample was 10 µl. The column's temperature was ambient. From the UV spectrum, rilpivirine and cabotegravir have a wavelength of 257 nm. At 257 nm, the analyte was seen to elute. The optimized chromatogram was displayed in **Fig. 3**.

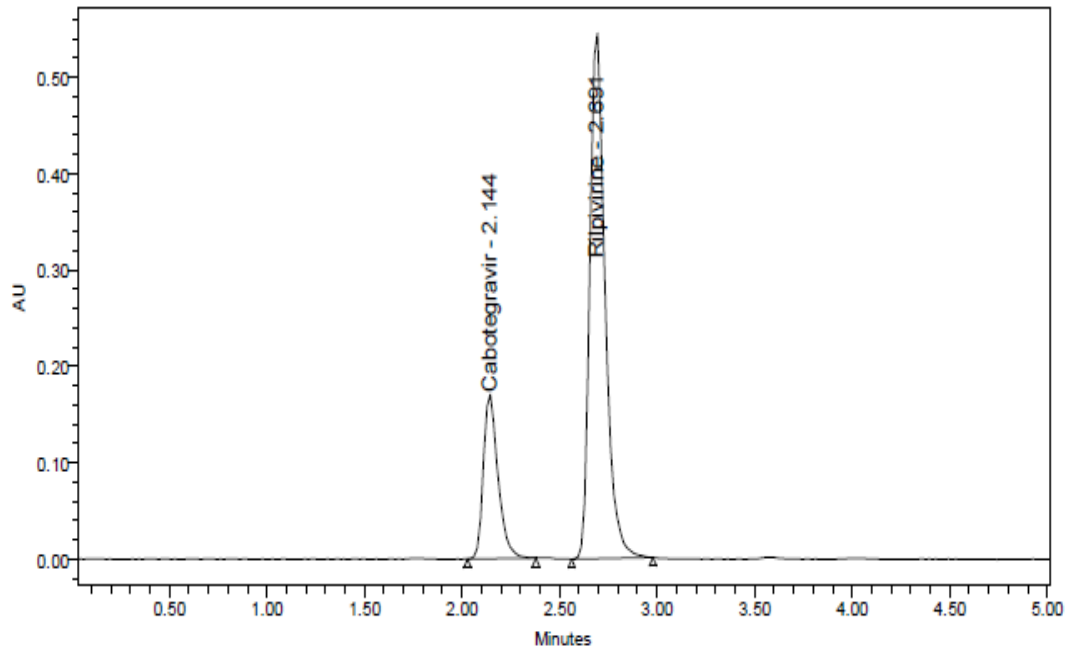

Fig. 3: Optimized chromatogram of cabotegravir and rilpivirine.

Method development

In Trial 1, a 50:50 mixture of water and methanol was used. Trial 2 was carried out employing a 50:50 ratio of OPA to methanol. In Trial 3, acetonitrile was used in a 50:50 ratio with 0.1N KH_2PO_4. Trial 4 was completed by utilizing a 75:25 ratio of acetonitrile to KH_2PO_4. Trial 5 involved optimizing the process using 0.1N KH_2PO_4: acetonitrile.

Method validation

System suitability parameters

The drug solution containing 10µg/ml was administered into the chromatographic system six times in duplicate, and the parameters pertaining to system suitability were ascertained. Notable were the peak tailing, the resolution, and the USP theoretical plate count.

Specificity

The HPLC system was loaded with samples in order to assess the specificity. The drug solution and the blank solution were contrasted. Interference between a blank response and a drug peak response in the output chromatograms was looked for.

Linearity

By adding diluents in varying quantities to the drug stock solutions of cabotegravir and rilpivirine, ranging from 12.5 - 75 µg/ml and 18.750 µg/ml - 112.500 µg/ml, respectively, different drug solutions were prepared to assess the linearity. The linearity plot was assessed using linear regression analysis.

Sensitivity

The LOD and LOQ were determined using the following formulas, which were based on the slope of the calibration and the standard deviation of the responses using different concentrations of the standard stock solution.

The limit of detection is equal to 3.3 times the standard deviation of the response or the slope of the analyte's calibration curve.

The quantification limit is equal to 10 x the standard deviation of the response / the slope of the analyte's calibration curve.

Accuracy

The standard stock solution was mixed with an acknowledged amount of rilpivirine and cabotegravir sample stock solution to determine accuracy at 50%, 100%, and 150%. The recoveries % were calculated.

Precision

Precision was investigated as intraday, interday, and system precision. Six distinct standard solution concentrations were injected on the same day in order to calculate intraday precision. The percentage RSD was calculated after measuring the peak area. Six distinct

standard solution concentrations were injected three times a week for three days in order to determine the inter-day precision. The percentage RSD was calculated after measuring the peak area.

Robustness

By injecting the samples and modifying the flow rate and mobile phase ratio, the robustness was ascertained.

Forced degradation studies

Oxidation

From the normal stock solution of rilpivirine and cabotegravir, 0.1 ml of the solution was pipetted out, and 20% of 1 ml H_2O_2 was added to it. The solutions were heated in a water bath for thirty minutes at 60°C. After cooling, the solutions were bring about to room temperature. The volume was adjusted with diluent. A 10 µl solution was introduced into the HPLC apparatus.

Acid degradation

1ml of the stock mixture of rilpivirine and cabotegravir was mixed with 1ml of 2N HCl. Then, it was refluxed for thirty minutes at 600°C. The resultant solution was made up with diluents and10 µl solution were injected into the system.

Alkali degradation

To 1ml of stock solution of rilpivirine and cabotegravir, 1 ml of 2 N NaOH was added and refluxed for thirty minutes at 60^0c. The sample's stability was determined by diluting the resulting solution with diluents. The system was injected with 10 µl, and the chromatograms were obtained.

Thermal degradation

The standard sample solution was heated at 105°C for six hours. Diluent was used to prepare the solution. After the system has cooled, 10 µl of the solution was added.

Photolytic degradation

By placing the beaker in the UV chamber for a day or 200 watt hours per square meter in the photo stability chamber, the 1500 µg/ml and 1000 µg/ml solutions were subjected to UV light, and 10 µl was injected into the system.

Assay

Cabenuva, bearing the label claim Cabotegravir 400mg, Rilpivirine 600mg/2ml was purchased. Assay was performed with the above dosage form. Six sample solutions of cabotegravir and rilpivirine were prepared and injected into the HPLC system. The developed method was applied to dosage form.

RESULTS AND DISCUSSION

Results

Method optimization of chromatographic conditions

Maximum absorbance of the drug was found at 257 nm by UV spectroscopy measurement. Following multiple trials with various mobile phases, a suitable and accurate HPLC approach was used for the analysis of cabotegravir and rilpivirine. The first trail started with water and methanol in the ratio of 50:50. This trail was not selected as only cabotegravir peak was eluted but not rilpivirine. The second trail was with orthophosphoric acid and methanol in the proportion of 50:50. This trail was not chosen as broad peak shape was observed for cabotegravir. The third trail was with acetonitrile and 0.1N KH_2PO_4 in the ratio of 50:50. This was not selected as both the peaks were eluted in void volume range. The fourth trail was with acetonitrile and KH_2PO_4 in the ratio of 75:25. This was not selected as both peaks were eluted with more elution time. The fifth trail was optimised by 0.1N KH_2PO_4 and acetonitrile in the ratio of 70:30 and the attained chromatogram was originated to be in good shape.

Method validation

System suitability parameters

The parameters for the rilpivirine and cabotegravir revealed that the theoretical plates were greater than 2000 and the tailing factor was less than 2. The Lab Solutions software present in the HPLC has a module for calculating the number of theoretical plates, tailing factor, and resolution. They are system generated results. **Table 1** displayed statistics on system appropriateness.

Table 1: System suitability parameters.

S. No	Rilpivirine			Cabotegravir			
Injection	Retention time (minutes)	Number of Theoretical plates	Tailing factor	Retention time (minutes)	Number of Theoretical plates	Tailing factor	Resolution
1	2.139	3856	1.3	2.688	6162	1.33	3.9
2	2.140	3792	1.3	2.692	6509	1.31	3.9
3	2.143	4261	1.2	2.692	6699	1.31	3.9
4	2.144	4120	1.2	2.694	6560	1.27	3.9
5	2.144	3780	1.3	2.694	6547	1.31	3.9
6	2.144	4163	1.3	2.695	5814	1.37	3.8

Linearity

Six linear concentrations of rilpivirine (18.750-112.500 µg/ml) and cabotegravir (12.5-75 µg/ml) were injected in a duplicate manner. Regression equations obtained for cabotegravir was y = 7596.9x + 1542.1 and of rilpivirine was y = 7517.8x + 5409.4. Correlation coefficient obtained was 0.999 for the two drugs. **Fig. 4 and 5** show the calibration curves of cabotegravir and rilpivirine, respectively. The linearity parameters were included in **Table 2**. Cabotegravir and rilpivirine LOD was found to be 0.25 and 1.79 µg/ml respectively and cabotegravir and rilpivirine LOQ was found to be 0.77 and 5.44 µg/ml.

Accuracy

Three injections were given for each degree of accuracy. The mean percent recovery for cabotegravir and rilpivirine, respectively, was found to be 100.06% and 99.46%. **Table 3 and 4** showed the accuracy data of cabotegravir and rilpivirine respectively.

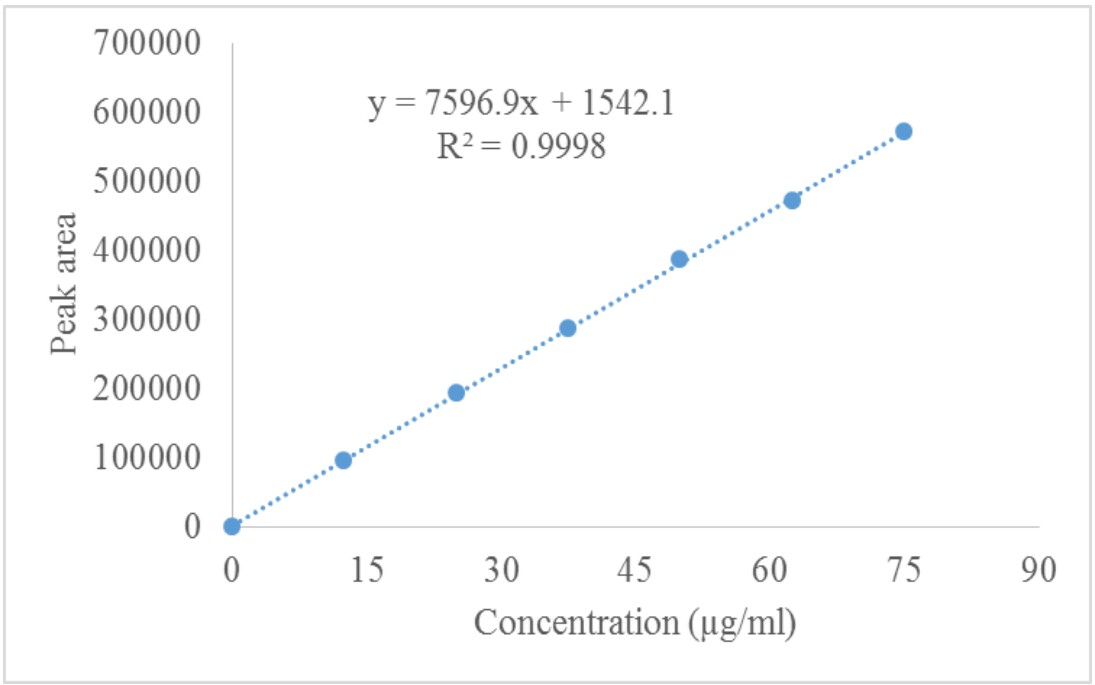

Fig. 4: Calibration curve of cabotegravir.

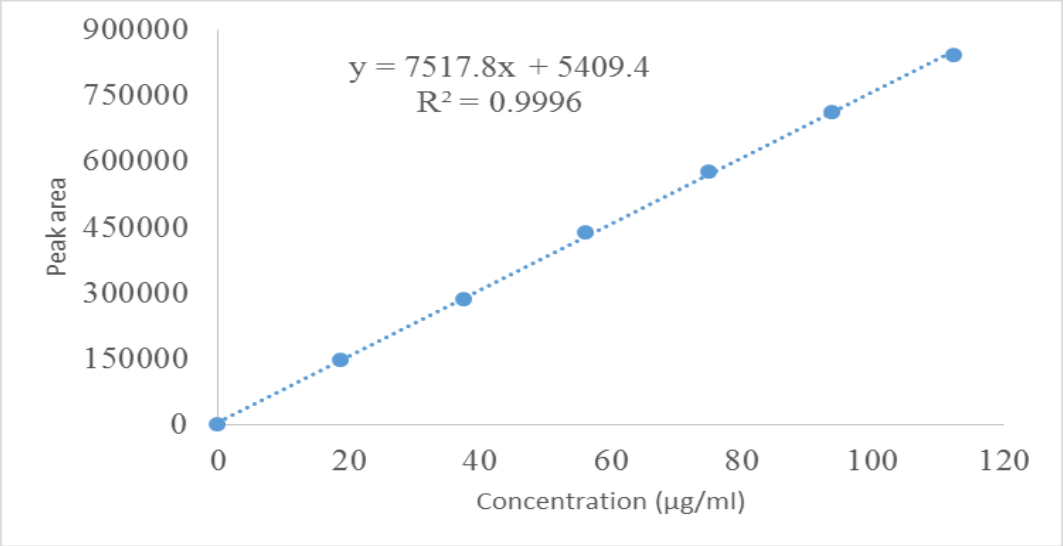

Fig. 5: Calibration curve of rilpivirine.

Table 2: Linearity parameters.

S. No		Cabotegravir	Rilpivirine
1	LOD	0.25 µg/ml	1.79 µg/ml
2	LOQ	0.77 µg/ml	5.44 µg/ml
3	R^2	0.9998	0.9996

Table 3: Accuracy data of cabotegravir.

Level %	Spiked amount (µg/ml)	Recovered amount (µg/ml)	Recovery %	% Recovery mean
50%	25	25.03	100.13	100.06%
	25	24.86	99.43	
	25	25.16	100.64	
100%	50	50.28	100.56	
	50	49.66	99.31	
	50	49.66	99.31	
150%	75	74.75	99.67	
	75	75.41	100.55	
	75	75.68	100.91	

Table 4: Accuracy data of rilpivirine.

Level %	Spiked amount (µg/ml)	Recovered amount (µg/ml)	Recovery %	% Recovery mean
50%	37.500	37.215	99.24	99.46%
	37.500	37.216	99.24	
	37.500	37.124	99.00	
100%	75.000	75.437	100.58	
	75.000	74.381	99.17	
	75.000	74.776	99.70	
150%	112.500	111.518	99.13	
	112.500	111.569	99.17	
	112.500	112.394	99.91	

Repeatability

Average area, SD, and percent RSD were calculated for rilpivirine and cabotegravir. They were found to have respective values of 0.6% and 0.7%. Data on repeatability are displayed in **Table 5**.

Intermediate precision

Chromatogram values for intermediate precision were found to be 0.4 and 1.2 for rilpivirine and cabotegravir respectively. The outcomes were displayed in **Table 6**.

Table 5: Repeatability of cabotegravir and rilpivirine.

S. No	Area of rilpivirine	Area of cabotegravir
1.	575550	381174
2.	574180	384892
3.	579531	388092
4.	571415	382926
5.	579234	383125
6.	572585	386929
Mean	575416	384523
S.D	3378.7	2622.3
%RSD	0.6	0.7

Table 6: Intermediate precision of cabotegravir and rilpivirine

S. No	Area of rilpivirine	Area of cabotegravir
1.	576855	382019
2.	578200	387307
3.	573546	389288
4.	579618	379754
5.	576488	387700
6	575064	381254
Mean	576629	384554
S.D	2164.6	4006.1
%RSD	0.4	1.0

Robustness

The RSD % of flow minus, flow plus, mobile phase -, mobile phase +, temperature + and temperature - were observed to be 0.4, 1.1, 0.4, 0.9, 0.4, 1% respectively for cabotegravir and 0.4, 0.3, 0.5, 0.4, 0.4, 0.4 for rilpivirine respectively. **Table 7** displayed an illustration of the results.

Specificity

There was no evidence of interference. **Table 8** displayed data regarding specificity. The blank chromatogram was displayed in **Fig. 6**.

Table 7: Robustness data of cabotegravir and rilpivirine.

S.no	Condition	%RSD of cabotegravir	%RSD of rilpivirine
1	Flow minus 0.9ml/min	0.400	0.400
2	Flow plus 1.1ml/min	1.100	0.300
3	Mobile phase 35A:65B	0.400	0.500
4	Mobile phase 25A:75B	0.900	0.400
5	Temperature 27°C	0.400	0.400
6	Temperature 33°C	1.000	0.400

Table 8: Specificity

S. No	Sample details	Retention time (min)
1	Blank solution	Interference was not detected
2	Cabotegravir	2.144 min
3	Rilpivirine	2.692min

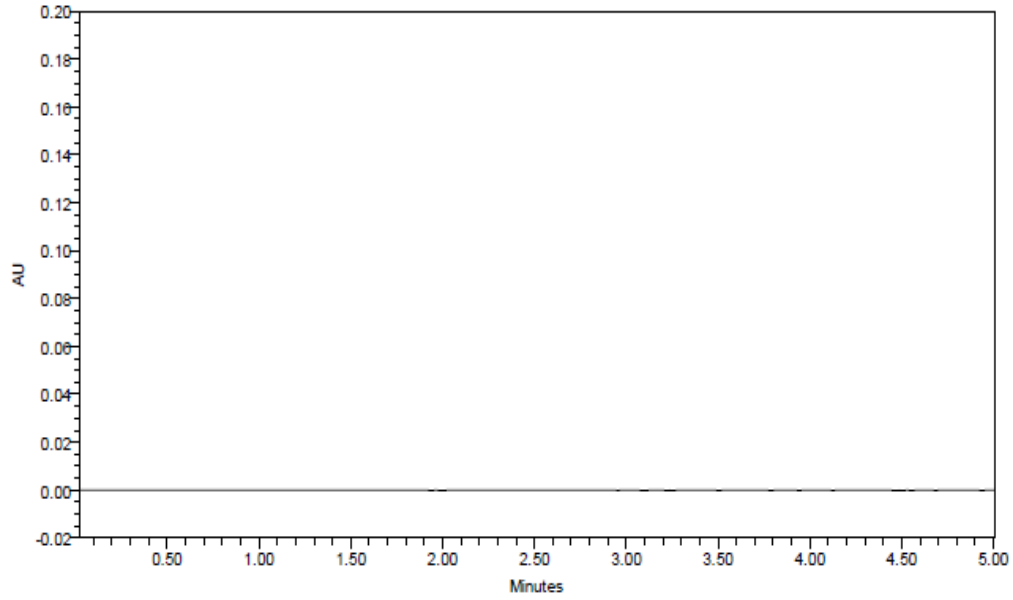

Fig. 6: Chromatogram of blank sample.

Sensitivity

Cabotegravir and rilpivirine LOD was found to be 0.25 and 1.79 µg/ml respectively and cabotegravir and rilpivirine LOQ was found to be 0.77 and 5.44 µg/ml.

Assay

Average % assay for rilpivirine and cabotegravir obtained was 99.93 % and 99.88 % respectively.

Forced degradation studies

Cabotegravir and rilpivirine were subjected to acid degradation (2.65%, 2.53%), base degradation (2.58%, 1.85%), peroxide degradation (4.42%, 4.77%), thermal degradation (2.63%, 2.67%), UV (1.38%, 1.27%), water (0.69%, 0.78%). The data from the forced degradation studies were shown in **Table 9.** The chromatograms for the various degradation categories were listed in **Fig. 7-12**.

Table 9: Forced degradation studies data of cabotegravir and rilpivirine.

Type of degradation	Rilpivirine			Cabotegravir		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	561265	97.47	2.53	374768	97.35	2.65
Base	565178	98.15	1.85	375038	97.42	2.58
Peroxide	548383	95.23	4.77	367966	95.58	4.42
Thermal	560461	97.33	2.67	374851	97.37	2.63
UV	568539	98.73	1.27	379659	98.62	1.38
Water	571364	99.22	0.78	382300	99.31	0.69

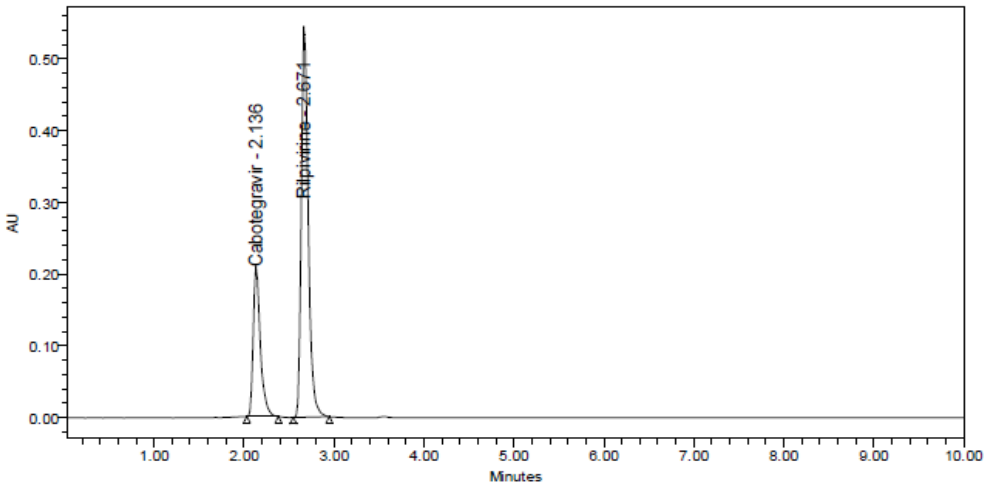

Fig. 7: Acidic degradation chromatogram of cabotegravir and rilpivirine.

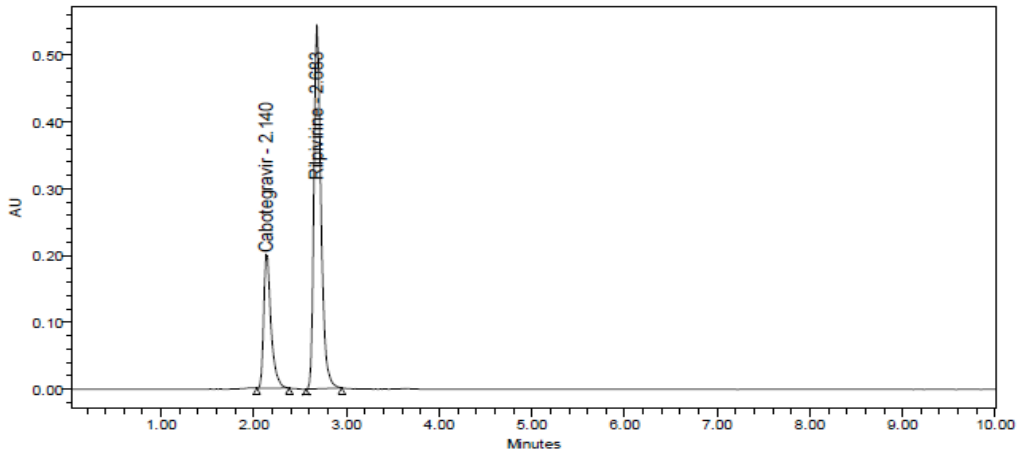

Fig. 8: Alkali degradation chromatogram of cabotegravir and rilpivirine.

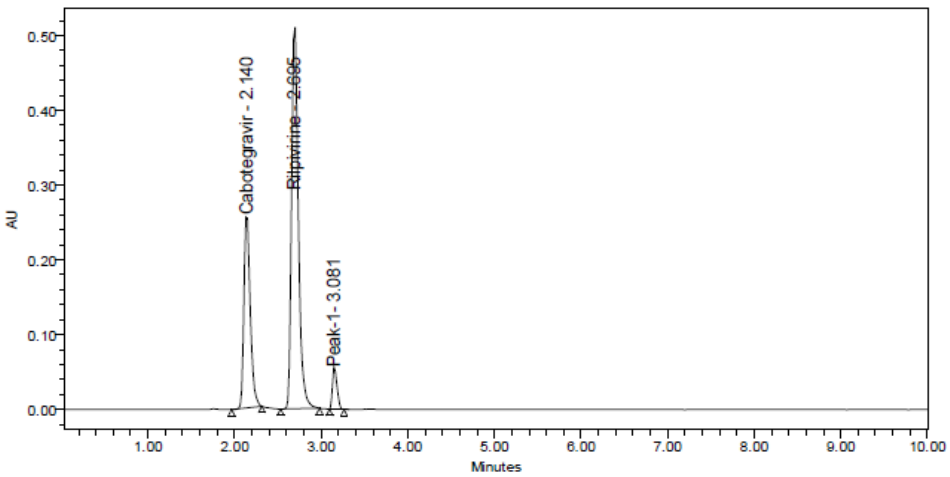

Fig. 9: Peroxide degradation chromatogram of cabotegravir and rilpivirine.

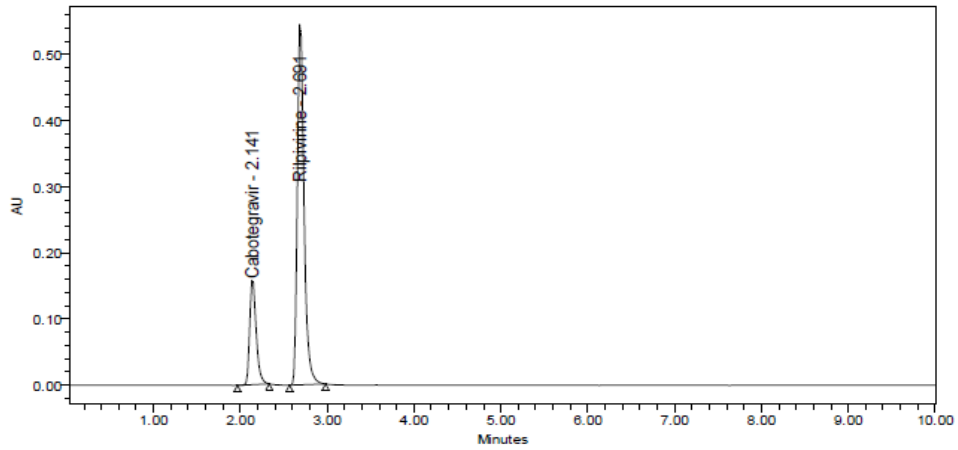

Fig. 10: Thermal degradation chromatogram of cabotegravir and rilpivirine

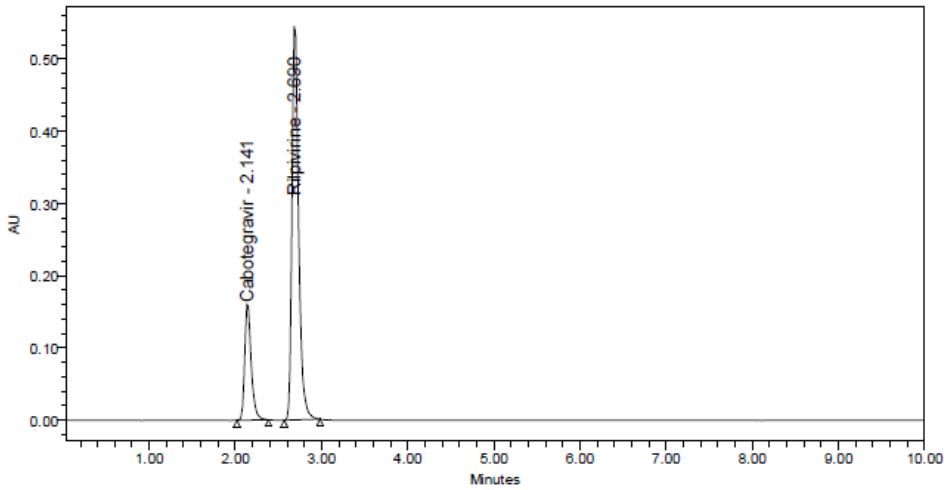

Fig. 11: UV degradation chromatogram of cabotegravir and rilpivirine

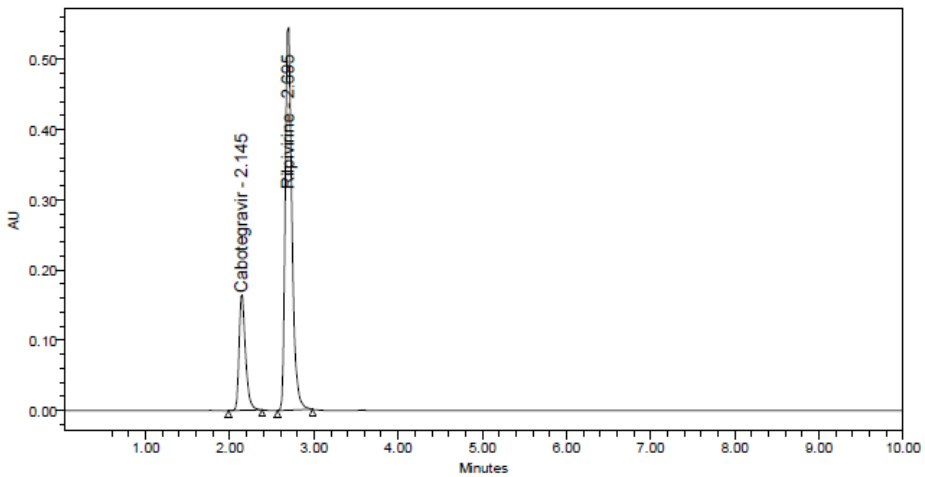

Fig. 12: Water degradation chromatogram of cabotegravir and rilpivirine.

Discussion

In accordance with ICH requirements, an RP-HPLC technology was developed and validated for the measurement of cabotegravir and rilpivirine in both pure and tablet dose forms. The Kromasil C18 column was used to separate the analyte. Mobile phase was in the ratio (70:30v/v) of 0.1N of KH_2PO_4 and

acetonitrile.1 ml/min was used as flow rate. The maximum absorbance of the drug was at 257 nm. A system suitability test was necessary for the method's development to make sure the system was suitable for the analysis of rilpivirine and cabotegravir. Before the samples from each day were analyzed, a proper procedure was set to guarantee that the HPLC equipment performed techniques that provided findings with an acceptable level of accuracy and precision. For rilpivirine, linearity was reached at a concentration range of 18.75-112.5 µg/ml and for cabotegravir, 12.5-75 µg/ml, using the suggested HPLC method. Acceptable linearity was demonstrated when the correlation coefficient was found to be within recognized bounds. Three levels of accuracy samples (50%, 100%, and 150%) were obtained using the traditional addition procedure. The excellent recovery rates of the method proved that the recommended strategy may be applied to quality control inspection. The chromatograms' repeatability was discovered to be within the predefined range (%RSD not exceeding 2.0 %). It demonstrated that the procedure might be repeated as a consequence. It was found that the chromatogram data with intermediate precision met the stipulated limit. It so proved that the process was found to be successful. Small, intentional adjustments were made to the chromatographic settings, such as the mobile phase flow rate (0.9 & 1.1 ml/min) and the ratios of 0.1N KH_2PO_4 and acetonitrile (65B:35A and 75B:25A). This allowed for the evaluation of robustness. It was found that the % RSD was robust. By injecting a blank solution to the HPLC system, specificity was tested. This demonstrated that at the typical cabotegravir and rilpivirine sample retention period, there was no interference in the blank sample. There was no interference with the blank sample at the normal cabotegravir and rilpivirine retention time. Thus, it is evident that the process was specific.

When determining LOD and LOQ, consideration was given to the following factors: LOD was defined as approximately S/N 3, LOQ as the lowest verified concentration with (%) RSD and (%) error 20%. It was found that LOD and LOQ were sensitive. The amount of drug content found in the sample solutions values fell within the permitted range of 90-110%, as stated on the label.

Cabotegravir and rilpivirine were subjected to acid degradation (2.65%, 2.53%), base degradation (2.58%, 1.85%), peroxide degradation (4.42%, 4.77%), thermal degradation (2.63%, 2.67%), UV (1.38%, 1.27%), water (0.69%, 0.78%). The results were less than 10% indicating that cabotegravir and rilpivirine were more resistant towards all forced degradation conditions applied. Additionally, the system suitability parameters did not exceed bounds.

Conclusion

Conferring to ICH standards, the suggested approach was validated for several parameters, including precision, linearity, accuracy, specificity, system appropriateness, and robustness. The obtained results satisfied the requirements for approval. Because the method is simple to use, accurate, affordable, and safe, it can be effectively used to the routine analysis of cabotegravir and rilpivirine in bulk and pharmaceutical dose forms.

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REFERENCES

1. A. Prasanna and S. Shobha Rani, "A validated stability indicating RP-HPLC method development for simultaneous estimation of cabotegravir and rilpivirine in pharmaceutical dosage form", ***World J Pharm Pharm Sci***, 10(10), 22-29 (2022).
2. V. Anuradha, T. Subrahmanyam, M. Raju, S. N. B. Murthy and A. K. Emmanuel, "Method development and validation for cabotegravir and rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR", ***Fut J Pharm Sci***, 7, 226 (2021).
3. N. Vasantha, C. Parthiban and M. Sudhakar, "RP-HPLC method development and validation for the simultaneous estimation of cabotegravir and rilpivirine in pharmaceutical dosage

form", ***Int J Pharm Pharm Sci***, 26 (1), 65-79 (2022).

4. I. K. Ramoller, M. T. A. Abbate, L. K. Vora, A. R. J. Hutton, KePeng, F. V. Zanutto, I. A. Tekko, K. Moffatt, A. J. Paredes, H. O. McCarthy and R. F. Donnelly, "HPLC-MS method for simultaneous quantification of the antiretroviral agents rilpivirine and cabotegravir in rat plasma and tissues", ***J Pharm Biomed Anal***, 213, 114698 (2022).
5. P. Yogi and P. Samixa, "RP-HPLC stability method development & validation for anti-HIV drugs cabotegravir & rilpivirine in I.M. injection and in human plasma", ***Int J Health Sci***, 6(S1), 9104-9117 (2021).
6. P. Courlet, S. A. Saldanha, M. Cavassini, C. Marzolini, E. Choong, C. Csajka, H. F. Günthard, P. André, T. Buclin, V. Desfontaine and L. A. Decosterd**,** "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 HIV**",** ***J Mass Spec*****,** 55(6), e4506 (2020).
7. K. S. Reddy, S. S. Shirsha and K. P. Kumar, "Validated stability indicating RP-HPLC method for the simultaneous estimation of rilpivirine and dolutegravir in bulk form", ***Int J Pharm Sci Res***, 12(6), 4991-4997 (2021).
8. E. D. Weld, T. L. Parsons, R. Gollings, M. McCauley, B. Grinsztejn, R. J. Landovitz and M. A. Marzinke, "Development and validation of a liquid chromatographic-tandem mass spectrometric assay for the quantification of cabotegravir and rilpivirine from dried blood spots", ***J Pharm Biomed Anal***, 228,115307 (2023).
9. M. Azeemuddin, H. K. Sharma and M. Z. Baba, "Method development and validation for the simultaneous estimation of cabotegravir and rilpivirine in bulk and pharmaceutical dosage form and stability studies by UPLC", ***Int J Food Sci Nutr***, 11(1), 2385-2391 (2022).

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تطوير طريقة تحليلية والتحقق من صحتها لتحديد متزامن للكابوتيجرافير والريبفيرين باستخدام التحليل الكروماتوجرافي عالي الأداء

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يمكن علاج النوع الأول من فيروس نقص المناعة البشرية عن طريق الجمع بين هذه الأدوية تم تطوير تقنية دقيقة ومحددة وجديدة وتم التحقق من صحتها من أجل التقييم المتزامن للكابوتغرافير والريبفيرين في التركيبات السائبة والطبية باستخدام التحليل الكروماتوجرافي عالي الأداء. كان الطور المتحرك بنسبة (٧٠:٣٠) الأسيتونتريل و ٠,١ بوتاسيوم ثنائي فوسفات الهيدروجين. تم استخدام معدل تدفق قدره ١ مل\دقيقة. كان الطول الموجي للكاشف ٢٥٧ نانومتر. وكان وقت التشغيل ٥ دقائق. تم العثور على قيم الحد الأدنى لكمية كابوتيغرافير وريلبيفيرين لتكون ٠,٧٧ ميكروغرام/مل و٥,٤٤ ميكروغرام/مل على التوالي. وقد ثبت أن النهج المقترح دقيق ومثالي للاستخدام في مختبرات مراقبة الجودة للفحص الكمي لأشكال الجرعات، المفردة والمختلطة.