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Research Article

Quality by Design Approach for RP-HPLC Method Development and Validation of Cabotegravir from Bulk Drug and Dosage Form

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ABSTRACT

A robust reversed-phase chromatographic method was developed for the analysis of cabotegravir in pure form and tablets. Using Design Expert software version 8, the technique was optimized through a central composite design with an emphasis on peak symmetry, theoretical plates, and retention time. A 50:50 ratio of mobile phase, consisting of acetonitrile and phosphate buffer (pH 4), at a flow rate of 1-mL/min, and a column oven temperature of 27.5°C were ideal parameters. Detection was performed at 256 nm using a BDS Hypersil™ C18 column and a PDA detector, with a run time of 10 minutes. The method's specificity, linearity, accuracy, robustness, and precision were all verified. Linearity was noted in the range of 10 to 50 µg/mL, with a correlation coefficient of 0.999. The %RSD for intraday and interday precision was 1.67 and 1.97%, respectively. Accuracy, as %recovery, was 100.70 at 80%, 98.91 at 100%, and 101.83 at 120% concentration levels. 1.8 µg/mL was the limit of detection (LoD), and 5.4 µg/mL was the limit of quantification (LoQ). All validation parameters were within the acceptable limits of the ICH Q2(R1) guideline. Forced degradation studies were conducted under acidic, alkaline, oxidative, photolytic, and thermal stress conditions. The drug demonstrated greater degradation in alkaline and oxidative conditions.

Introduction

Cabotegravir (CAB), classified as an antiviral agent and structurally analogous to dolutegravir, was approved by the USFDA, Health Canada, and the European Medicines Agency in 2020 and 2021.[1] The mechanism of action of CAB involves the inhibition of integrase, an essential enzyme that aids in the integration of the viral genome into the host genome. By obstructing this strand transfer, CAB effectively impedes the process of viral replication. As a result, the virus's ability to replicate is hindered, preventing it from spreading further. Given that the injectable solution is administered once a month and the oral pill is taken daily, it has a prolonged duration of effect.[2] Antiretroviral medications function primarily by decreasing the viral load of HIV in the bloodstream. Although cabotegravir does not serve as a treatment for individuals already infected with HIV, it possesses the capacity to diminish the likelihood of acquiring the virus, as well as mitigate the risk of developing additional HIV-related afflictions, which can include malignancies and severe infections.[3] The chemical structure of cabotegravir is shown in Fig. 1. Additionally, the FDA approved Vocabria, a tablet version of cabotegravir, which is intended to be taken in conjunction with oral rilpivirine (Edurant) for one month before initiating therapy.[4,5] Viiv Healthcare produces CAB, which has also been demonstrated to be a successful pre-exposure prophylactic.

The concept of quality-by-design (QbD) is articulated by the International Council for Harmonization (ICH) as a structured methodology for development, which is initiated with clearly defined objectives. This methodology

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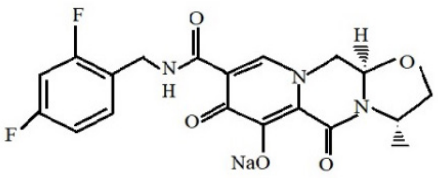


Fig. 1: Structure of cabotegravir

places an emphasis on understanding and controlling both products and processes through the application of sound scientific principles and quality risk management strategies.[6]

Integral to the QbD framework is the necessity for a comprehensive understanding of product and process dynamics, which involves the implementation of quality risk management and control strategies. Such a systematic approach not only enhances the assurance of product quality but also provides greater regulatory flexibility and promotes opportunities for ongoing improvements.[7] Furthermore, the principles of analytical methods are developed using quality by design, particularly through its variant known as analytical quality by design (AQbD). This adaptation reinforces the foundational elements of QbD while addressing the specific needs associated with the development of analytical methods.[8] This approach facilitates the development of analytical methods that are both robust and economically viable, ultimately leading to the creation of a design area that can be operated by a method through careful consideration of method-related factors.[9] In the context of pharmaceutical analysis, various factorial experimental designs, including central composite design (CCD), are commonly exploited to optimize the analytical process.[10] In the given manuscript, a CCD experimental design was used; the association between different factors and their corresponding outcomes is established, yielding statistically significant values. The experimental design encompassed the identification of three independent variables: the flow rate and the buffer's pH used in the mobile phase employed during the process, and the temperature maintained within the column. In contrast, the dependent variables were characterized by the number of theoretical plates (NTP), retention time, and peak symmetry observed. The method's validation was conducted as per established guidelines as stipulated in ICH Q2 (R1) and ICH Q14. [11-12]

A literature review indicates the use of a UV-visible spectrophotometer for the detection and quantification of cabotegravir. UV-vis lacks stability-indicating capability and separation efficiency, as it cannot differentiate CAB from its degradation products or impurities. In contrast, RP-HPLC enables effective separation and quantification, making it the preferred method for pharmaceutical quality control (QC) over UV-vis, which is mainly used for preliminary analysis.[13] Kovac *et al.* developed an analytical method utilizing UHPLC-MS for the identification of cabotegravir degradation products. However, the stability of separation efficiency under gradient elution conditions was inconsistent. Additionally, the UHPLC-MS system incurs significantly higher costs, limiting its accessibility for routine analytical applications. While several studies have reported on the combination of cabotegravir and pilpivirine, biological matrices, such as rat plasma, can induce ion suppression or enhancement in MS detection, potentially impacting accuracy.[14] Human plasma analysis may introduce matrix interferences, whereas RP-HPLC for tablets provides a cleaner matrix and simpler quantification.[15-18] However, none of the described methods focus on A QbD approach for bulk drug and its dosage forms, i.e., central composite design. It ensures regulatory compliance with ICH Q8-Q14 by enabling a well-defined operational design space that facilitates seamless method transfer across laboratories and instruments. Proactive risk assessment enhances method control, thereby reducing out-of-specification (OOS) results. Statistical optimization minimizes rework, lowering development costs & risk-based method development, reducing approval time.

Materials And Methods

Materials

Cabotegravir was obtained as a gift sample. The study's other chemicals and reagents were all analytical grade, while the solvents utilized conformed to HPLC specifications. The assay was conducted using the commercially available formulation, Vocabria tablet 30 mg, produced by Viiv Healthcare Ltd.

Instrument and References

The RP-HPLC Jasco Extrema LC System 4000 series with the photodiode array detector. BDS Hypersil^{TM} C_{18} column was used.

Chromatographic Conditions

Analytical methodology employs a BDS HypersilTM C18 column characterized by the following dimensions: length, 250 mm; diameter, 4.6 mm; and particle size, 5.0 µm. To achieve optimal performance, the column was equilibrated using a mobile phase composed of 50:50 (v/v) acetonitrile and phosphate buffer in equal proportions. The buffer's pH was meticulously well-adjusted to 4.0 by adding glacial acetic acid. Operational parameters included a flow rate established at 1-mL/min, along with a consistent column temperature maintained at 27.5°C. The elution process was facilitated by a PDA detector, which was calibrated to monitor absorbance at a wavelength of 256 nm. An injection volume of 10 µL was utilized in this protocol. The chromatographic conditions described above contributed to the effective separation of cabotegravir while ensuring optimal peak symmetry.

In the optimization of HPLC method, various parameters were systematically examined. Specifically, the pH of the buffer, temperature, and flow rate were analyzed as three independent variables, each assessed at three distinct levels through the application of CCD. This approach facilitated a comprehensive evaluation of the methods performance.

Preparation of Standard stock solution

A precise measurement of cabotegravir (100 mg) was placed in a 100 mL flask, which contained acetonitrile. Subsequently, acetonitrile was added to adjust the volume, resulting in the formation of a stock solution (standard) with a concentration 1000 µg/mL. Following this preparation, volume of 10 mL was transferred from this solution into another 100 mL flask, by utilizing distilled water to adjust the volume of this flask, thereby yielding a standard stock solution with a concentration of 100 µg/mL. Additionally, dilutions had been made from the solution containing 100 µg/mL, employing a diluent mixture comprised of 90:10 v/v water:acetonitrile.

Selection of Wavelength

The cabotegravir standard solution (10 µg/mL) was scanned under a UV-visible Spectrophotometer from 200 to 400 nm against Water: Acetonitrile (90:10) as a blank solution to determine analytical wavelength. For selecting the λ_{max} standard solution of cabotegravir was scanned in the spectrum mode.

HPLC Method Development by QbD Approach

The following summarizes how the analytical quality-by-design principles were applied in the HPLC method development is as follows:

Selection of quality target product profile (QTPP)

The QTPP serves an essential role in identifying variables that influence its parameters. In the context of the proposed HPLC method, it has been determined that NTP, peak symmetry, and retention time are integral components of QTPP. These factors collectively contribute to the overall assessment and optimization of the analytical method.

Determine critical quality attributes (CQAs)

CQAs represent methodological parameters that exert a direct influence on the quality target product profile. Among these parameters, significant factors include the buffer's pH, the flow rate of the mobile phase, and the temperature of the column oven, all of which require meticulous regulation. Maintaining these parameters within specific ranges is crucial for ensuring that the QTPP remains aligned with acceptable thresholds. Consequently, the deliberate management of these factors is essential to uphold the integrity of the QTPP response.

Factorial design

The CQAs and QTPP were identified, and then a central composite design was used. This methodological approach aimed to optimize and identify three significant parameters: the buffer's pH, the column temperature, and the flow rate of the HPLC. This design facilitated an examination of both interaction effects and quadratic effects among the aforementioned parameters in relation to key performance indicators, including retention time, theoretical plates, and peak symmetry factor. The central composite statistical screening design served as the analytical framework for this study. A three-factor experimental design has been employed to analyze the effects of specific variables affect quadratic response surfaces. Design incorporated the parameters of buffer solution pH, column temperature, and flow rate, each evaluated at three distinct levels. The statistical analysis was conducted utilizing Design Expert (Version 8) software, allowing for the optimal fitting of second-order quadratic polynominal is expressed as follows:

$$Y = \beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{11}A_2+\beta_{22}B_2+\beta_{33}C_2+\beta_{12}AB+\beta_{13}AC+\beta_{23}BC$$

In this context, the variables designated as A, B, and C serve as independent factors, each assigned specific codes corresponding to various levels of influence. Conversely, Y symbolizes the dependent response that is assessed for each possible combination of these factor levels. The term β_0 refers to the intercept, while the coefficients β_1 through β_{33} represent the regression parameters determined through the experimental data pertaining to Y. The terms AB, BC, AC represents interaction effects and A^2, B^2, C^2 denote the quadratic terms. The examination of multi-variable interactions among variables and process parameters has led to selection of specific factors based on preliminary analyses. The independent variables identified for this study include the pH of the buffer solution, temperature of the column, and flow rate. In contrast, the dependent variables are defined as peak symmetry, NTP, and retention time, which correspond to the aforementioned independent variables.

Analysis of experimental results and determination of optimal method conditions

Evaluating the conditions of the method was conducted utilizing CCD approach. This evaluation initially centered on parameters such as peak symmetry, NTP, and retention time. Cabotegravir analysis has facilitated the determination of specific chromatographic conditions essential for optimal measurement. It was established that the acceptable ranges, defined from robust regions, indicate that deliberate alterations in method parameters do not comprise the quality of the results obtained. This is crucial for ensuring the method's reliability during subsequent validation tests.

In cases where the modelling experiments yield responses that fall outside the desired parameters, it becomes necessary to optimize the relevant variables at various

levels until acceptable responses are achieved. The optimization of the most suitable chromatographic conditions will be facilitated through the utilization of Design Expert tools, thereby ensuring rigorous refinement of the methodology.

Forced Degradation Studies

Studies of forced degradation, sometimes referred to as stress testing, were conducted in accordance with ICH guidelines Q2 (R1) to assess the degradation susceptibility of cabotegravir and confirm that the developed stability-indicating analytical method is specific. The drug was exposed to a range of stress conditions, including oxidative, alkaline, and acidic environments, by treating a cabotegravir solution (100 µg/mL) with 30% hydrogen peroxide, 1 N NaOH, and 1 N HCl, respectively. Additionally, thermal stability was evaluated by exposing the bulk drug powder to 110°C in a hot air oven for one hour, while photostability was assessed by subjecting the drug to UV radiation in a photostability chamber for 2 hours.

Analytical Method Validation

The validation process for analytical methods necessitates the compilation of documented evidence that affirms a substantial degree of confidence in the efficacy of a specified method. This validation ensures that the employed procedure is suitable for its designated application. Following the guideline stipulated in ICH Q2 (R1), an HPLC method established for estimating cabotegravir has undergone a rigorous validation process.

Specificity

The specificity of the method was evaluated through the analysis of chromatograms from injections of diluent, blank, and mobile phase samples. To ensure the purity of the cabotegravir peak in these samples, a photodiode array detector was used to scan wavelengths between 200 to 400 nm.

Linearity

The evaluation of cabotegravir involved an assessment of linearity through the analysis of six distinct concentration levels, specifically ranging from 10 to 50 µg/mL. Subsequently, a calibration curve was constructed by plotting the concentration on the x-axis against the corresponding peak area on the y-axis. Thereafter, the correlation coefficient values and regression equation were calculated to quantify the relationship between the variables assessed.

Precision

Precision study was conducted through the implementation of interday and intraday analyses. The evaluation of both precision types involved assessing a 50 µg/mL concentration over a period of two days, and the %RSD was calculated. The criterion for accepting the %RSD is a value below 2.

Accuracy

An evaluation of the accuracy of the given analytical method was conducted through a recovery study on a commercially available formulation. This assessment involved the application of three distinct levels of standard addition, specifically at 80, 100, 120%. The %recovery of cabotegravir was then calculated. As per ICH guidelines, the acceptable %recovery range is 98 to 102% of the standard edition.

LoD & LoQ

The limit of detection (LoD) refers to the minimal concentration level of a substance that can be reliably identified as well as differentiated from the surrounding baseline noise. The limit of quantification (LoQ) denotes the minimal concentration level of a substance that could be consistently quantified with precision. The ICH guidelines stipulate specific equations for the evaluation of both LoD and LoQ. These equations articulated as: LoD = 3.3 × σ/SD and LoQ = 10 × σ/SD. In these equations, σ represents the standard deviation associated with the y-intercept, and SD signifies the slope of the calibration curve. Thus, a clear distinction exists between the two limits, wherein the LoD focuses on detection capabilities, whereas the LoQ emphasizes the reliability of quantification.

Robustness

The method's stability was assessed through an evaluation process that involved implementing slight modifications to specific parameters, including flow rate, temperature, and wavelength. Through a systematic alteration of these conditions, assessments of the method's reliability and consistency were conducted.

System Suitability

An assessment of system suitability was conducted through the execution of six replicate analyses involving cabotegravir. This evaluation involved calculating the retention time, peak symmetry, and theoretical plates for the standard solutions.

Result And Discussion

In the initial phase of the experimental procedure, a mobile phase consisting of methanol and acetonitrile in a volumetric ratio of 20:80 was used; however, this configuration resulted in an excessively prolonged retention time. Subsequent experiments employing various ratios of water and acetonitrile—specifically, 20:80 v/v, 30:70 v/v, 40:60 v/v, and 50:50 v/v—failed to yield distinct peaks, thereby indicating a need for further refinement. To address these challenges, a distinctive mobile phase comprising acetonitrile and phosphate buffer in a volumetric proportion of 60:40 was implemented. Modifications to the chromatographic parameters, including the buffer's pH, flow rate, and

column oven temperature, were conducted with the objective of enhancing both peak shape and symmetry. These adjustments were systematically executed to ensure compliance with system suitability test criteria under improved conditions. Ultimately, the mobile phase was optimized to an equal volumetric ratio (50:50 v/v) of acetonitrile and phosphate buffer, with the pH precisely adjusted to 4.0 by incorporating glacial acetic acid. The optimization process benefited from the application of a central composite design methodology, which provided a structured framework for the refinement of diverse variables within the specified design domain.

HPLC Method Development by QbD Approach

Quality target product profile

The optimization of HPLC chromatographic conditions was guided by identifying a defined QTPP, which encompassed parameters such as peak symmetry, retention time, and NTP.

Critical quality attributes

The identification of the buffer solution's pH, column oven temperature, and mobile phase flow rate has been recognized as critical quality attributes (CQAs).

Factorial design

The proposed HPLC method was developed utilizing a central composite design (CCD). Table 1 presents a comprehensive summary of the optimization parameters.

Design space

The application of CCD constituted the core of the response surface method, which employed a quadratic model across 20 experimental runs. This methodological framework facilitated the examination of multiple variables, including the buffer's solution pH, the temperature of the column oven, and the flow rate. These factors were analyzed in connection with the 3 designated response factors: peak symmetry, NTP, and retention time. Subsequently, the outcomes derived from this analysis were organized into a systematic summary.

The analysis of the retention time, as illustrated in Fig. 2 and designed by the expression: Retention time (for corresponding actual values) = $13.623 + 0.3663 \times A + 0.1510 \times B - 21.773 \times C - 7.500 \times AB - 0.0250 \times AC + 0.0200 \times BC - 0.01704 \times A^2 - 2.9090 \times B^2 + 9.309 \times C^2$, indicates several key relationships between the variables involved. Specifically, the coefficient associated with the pH of the buffer (A), denoted as β1 (+0.3663), exhibits a positive

Table 1: Optimization of parameters for the analysis of cabotegravir using CCD

Runs	*Factor-1* *A: pH (±2)*	*Factor-2* *B: Temp. (^{0}C) (±2.5^{o}C)*	*Factor-3* *C: flow rate (±0.75)*	*Response-1* *Rt*	*Response-2* *Theoretical plate*	*Response-3* *Peak symmetry*
1	2.0	27.50	0.75	5.1	7723	1.4
2	6.0	30.00	1.00	3.8	8126	1.3
3	6.0	25.00	1.00	3.9	7188	1.5
4	2.0	30.00	1.00	3.8	5514	1.4
5	2.0	25.00	1.00	3.9	7364	1.3
6	4.0	27.50	0.50	7.6	9327	1.3
7	4.0	25.00	0.75	5.2	9562	1.1
8	6.0	25.00	0.50	7.7	11576	1.5
9	2.0	25.00	0.50	7.5	7971	1.5
10	6.0	27.50	0.75	5.1	9388	1.3
11	4.0	27.50	0.75	5.2	9562	1.1
12	4.0	27.50	0.75	5.2	9562	1.1
13	4.0	27.50	0.75	5.2	9562	1.1
14	2.0	30.00	0.50	7.5	7901	1.5
15	4.0	30.00	0.75	5.1	9180	1.1
16	4.0	27.50	0.75	5.2	9562	1.1
17	6.0	30.00	0.50	7.4	9395	1.6
18	4.0	27.50	1.00	3.9	7786	1.1
19	4.0	27.50	0.75	5.2	9562	1.1
20	4.0	27.50	0.75	5.2	9562	1.1

Table 2: Optimized condition obtained from QbD

pH of buffer	*Temperature of column*	*Flow rate*	*Retention time*	*Theoretical plates*	*Peak symmetry*	*Desirability*
4.00	27.50	1ml/min	3.9	7675	1.1	1.000

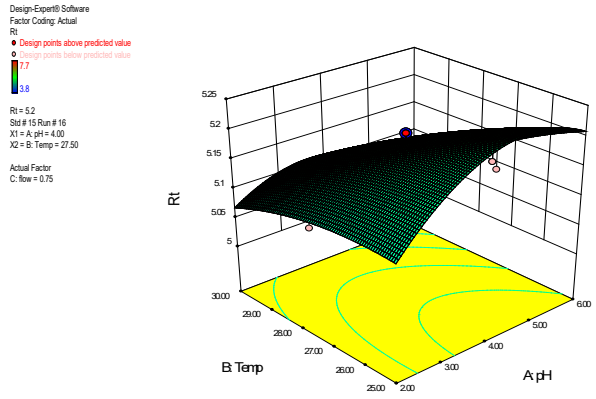


Fig. 2: 3D surface plot analyzing the impact of variables on cabotegravir's R1 retention time using CCD

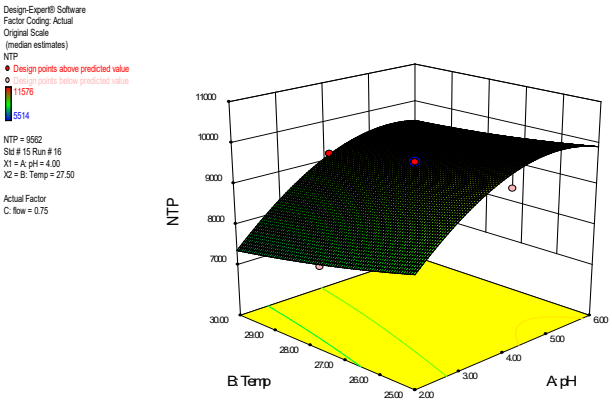


Fig. 3: 3D surface plot for effect of factors on R2 number of theoretical plates of cabotegravir by using CCD

correlation with the retention time. Consequently, an increase in pH results in an elevation of the retention time. Similarly, the temperature of the column (B), represented by β2 (+0.1510), also contributes positively to retention time, suggesting that higher temperature corresponds to longer retention time. In contrast, the flow rate (C), indicated by the negative coefficient β3 (-21.773), reveals an inverse relationship with retention time. As the flow rate increases, a substantial decline in the retention time frame occurs, highlighting the significant effect of this parameter on the overall retention time.

The analysis of the number of theoretical plates, as illustrated in Fig. 3 and equation number of theoretical plates (for actual values) = 145.26 + 8.2111 × A − 5.2862 × B +59.223 × C + 0.1662 × AB−2.8738 × AC+0.9723 × BC−1.0144 × A^2 + 0.0567 × B^2 − 64.586 × C^2, it was suggested that β_1 (+8.2111) & β_3 (+59.223) positive coefficients indicates

Table 3: Results of forced degradation studies

Conditions	*%Degradation*	*%Recovery*
Acidic (1N HCl)	0.53	99.46
Alkaline (1N HCl)	4.21	95.79
Thermal (110°C)	4.07	95.92
Oxidative (30% H_2O_2)	5.91	94.09
Photolytic (UV Chamber)	3.69	96.31

Table 4: Linearity of cabotegravir

S. No	*Concentration (µg/mL)*	*Peak area*
1	10	171065
2	20	408723
3	30	635191
4	40	860053
5	50	1124046
Correlation coefficient equation		y = 23572x−67372
r^2		0.999
Slope		23572

that as the buffer solution pH (A) & flow rate of mobile phase (C) both increase respectively, then there is a corresponding increase in the NTP. β_2 (−5.2862) negative coefficient indicates that an increase in temperature (B) leads to a decrease in the NTP.

The analysis of the peak symmetry, as illustrated in Fig. 4 and expression of peak symmetry (for corresponding actual values) = 1.8450 − 0.3575 × A + 0.0500 × B − 1.6200 × C −5.000 × AB + 1.5700 × AC −0.0400 × BC + 0.0625 × A^2 − 7.1909 × B^2 +1.600 × C^2, it concludes that β_1 (−0.3575) & β_3 (−1.6200) the negative coefficients suggests that as the pH of the buffer(A) & the flow rate(C), respectively, peak symmetry decreases. β_2 (+0.0500) the positive coefficient indicates that as the temperature (B) increases, peak symmetry decreases.

Optimized condition obtained

The analysis involved a thorough evaluation of all responses recorded under different experimental conditions using the design expert (version 8). Table 2 presents optimized HPLC conditions along with the corresponding predicted responses. To verify the accuracy of the predicted values, the observed values for each response were determined by running HPLC chromatograms with specific buffer solution pH, column temperature, and flow rate. These values observed were then subsequently contrasted with the anticipated outcomes to calculate percentage of predicted error.

Table 5: Interday and Intraday precision of Cabotegravir

Interday Precision			*Intraday Precision*		
Sr. No.	*Sample name*	*Peak area*	*Sr. No.*	*Sample name*	*Peak area*
1	Test 1	1165540	1	Test 1	1110676
2	Test 2	1143934	2	Test 2	1130864
3	Test 3	1110618	3	Test 3	1118742
4	Test 4	1126300	4	Test 4	1166688
5	Test 5	1135288	5	Test 5	1150756
6	Test 6	1141384	6	Test 6	1157306
Mean		1137177	Mean		1139172
S.D.		18412.35	S.D.		22443.67
%RSD		1.62	%RSD		1.97

Forced Degradation Study

Cabotegravir exhibited varying degrees of degradation under different stress conditions. In an acidic environment (1 N HCl), the drug exhibited minimal degradation of 0.53%, with a high recovery of 99.46%. However, under alkaline conditions (1 N NaOH), it underwent significant degradation, resulting in a 4.21% loss, with a corresponding recovery of 95.79%. Thermal stress at 110°C lead to a 4.07% degradation, maintaining a recovery of 95.92%. Exposure to oxidative conditions (30% H_2O_2) resulted in the highest degradation, reaching 5.91%, with a corresponding recovery of 94.09%, indicating its susceptibility to oxidative stress. Photolytic degradation under UV light was comparatively lower at 3.69%, yielding a recovery of 96.31%. These results suggest that cabotegravir is more vulnerable to degradation in alkaline and oxidative environments than in acidic or photolytic conditions. Table 3. represents the results of forced degradation studies & Figure 5. illustrates the forced degradation study chromatograms.

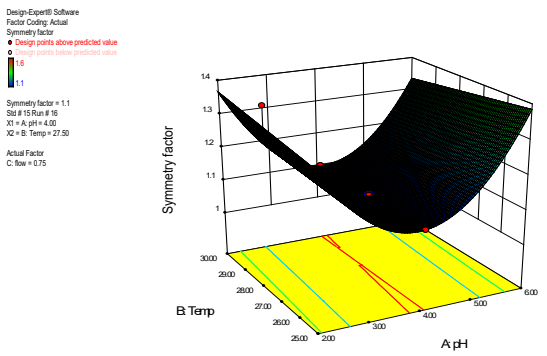


Fig. 4: 3D surface plot for the effects of factors on R3 peak symmetry by using CCD

Method Validation

Specificity

A blank sample composed of the diluent was injected to evaluate potential interference at the analyte's retention time. The results indicated no interference from contaminants, degradants, or matrix components, as demonstrated in Figure 6. These findings confirm the specificity of the analytical method.

System suitability

A system suitability assessment was performed on a typical chromatogram to assess key variables. The observed retention time was determined to be 3.9 minutes, with the NTP being 7675.39, & the peak symmetry was recorded at 1.12. These parameters collectively contribute to the assessment of the chromatographic performance. Fig. 7 illustrates the 3D surface plot that depicts the desirability associated with the attainment of an optimized formulation.

Linearity

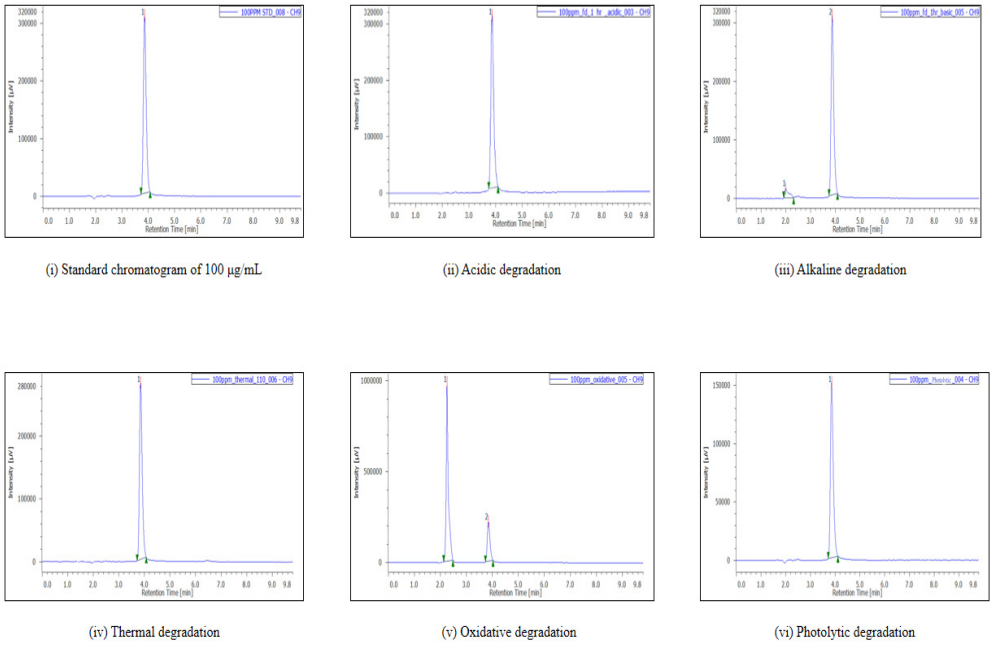
A linear relationship was demonstrated by the calibration curve established for cabotegravir across a concentration

Table 6: Accuracy of cabotegravir

Level	*Total conc (µg/mL)*	*Peak area*	*%Recovery*	*Mean conc. (µg/mL)*	*Standard deviation*	*%RSD*
	24	500325				
80%	24	504460	100.70%	24.157	0.0903	0.37
	24	501510				
	30	663043				
100%	30	649985	98.91%	30.794	0.3142	1.02
	30	662579				
	36	800476				
120%	36	801098	101.83%	36.879	0.0892	0.24
	36	804391				

Table 7: Robustness studies

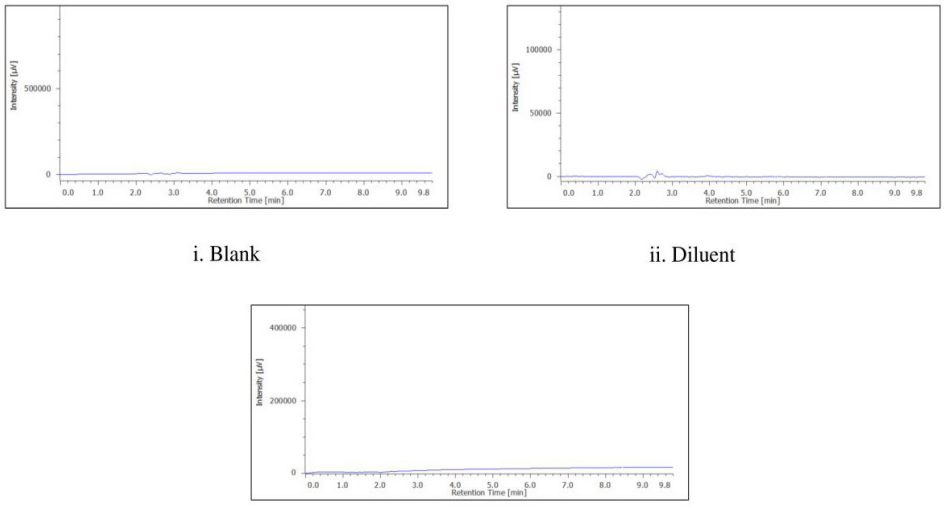
S. No			*1*	*2*	*3*	*MEAN*	*SD*	*%RSD*
Flow rate	0.8 mL/min	AREA	1175212	1181012	1187763	1181329	6281.50	0.53
	1.2 mL/min	AREA	795506	781568	775946	784340	10070.32	1.28
Temp.	22.5°C	AREA	815562	823132	802855	813850	10246.38	1.26
	32.5°C	AREA	838429	859637	856408	851491	11426.94	1.34
Wavelength	254 nm	AREA	1125172	1122024	1102310	1116502	12391.01	1.11
	258 nm	AREA	1003524	1008191	1001119	1004278	3595.79	0.36



(i) Standard chromatogram of 100 µg/mL (ii) Acidic degradation (iii) Alkaline degradation

(iv) Thermal degradation (v) Oxidative degradation (vi) Photolytic degradation

Fig. 5: Forced degradation studies chromatograms for cabotegravir



i. Blank ii. Diluent

iii. Mobile phase

Fig. 6: Specificity chromatogram for blank, diluent, & mobile phase of cabotegravir

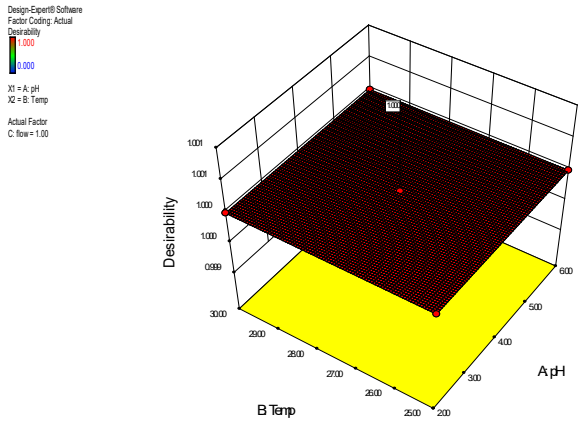


Fig. 7: 3D surface plot depicts the desirability for optimized formulation

of 10-50 µg/mL, as presented in Table 4. The regression equation derived from this calibration curve was y = 23573x−67372. This equation yielded a correlation coefficient (r^2) = 0.999 when the concentration was plotted against the peak area. A standard chromatogram of the linearity is depicted in Fig. 8.

Precision

Precision between days (inter-day) and within a day (intra-day) for cabotegravir was evaluated through six measurements of the same concentrations, i.e., 50 µg/mL, resulting in %RSD values of 1.62 and 1.97, respectively. Detailed results pertaining to interday and intraday precision are presented in Table 5. The observation of %RSD is below two suggesting that the method developed was found to be precise.

Accuracy

A study focused on recovery was carried out to evaluate the accuracy of the proposed HPLC method. To prepare the sample solutions, analytes were introduced at levels of 80, 100, and 120%. The %recovery results, shown in Table 6, ranged from 98 to 102%, demonstrating that the method complies with the accuracy requirements of ICH Q2 (R1) guidelines. Percent recovery values were within the acceptable range.

Robustness

Robustness studies were conducted using a 50 µg/mL solution of cabotegravir. Slight variations were implemented in several parameters, including adjustments to the flow rate, which was modified by ±0.2 mL/min, temperature, altered by ±5°C, and wavelength, which experienced a variation of ±2 nm. The results of these modifications are detailed in Table 7. Notably, the percentage relative standard deviation (%RSD) was maintained below 2 in response to these parameter adjustments, thereby indicating a level of robustness in the analytical method employed.

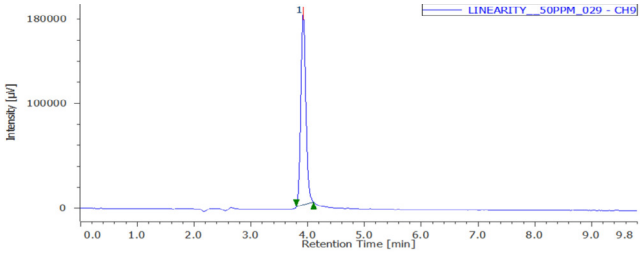


Fig. 8: Standard chromatogram of cabotegravir

LoD & LoQ

The detection limit (LoD) and quantification limit (LoQ) for cabotegravir were determined to be 1.8 and 5.4 µg/mL, respectively. This determination was derived from the evaluation of the standard deviation associated with both the intercept and slope of the relevant calibration curve.

Conclusion

A quality-by-design (QbD) methodology was employed in developing an HPLC method for cabotegravir, aiming to optimize critical parameters such as buffer pH, temperature, and flow rate. The method's objective was aligned with the analytical target product profile, and a CCD was employed to explore the interrelationships of these factors at three different levels. This systematic approach enabled the scouting and optimization of method components, resulting in a deeper understanding of how each factor affects chromatographic separation. The identification of the best system and the ultimate design space was achieved through a multivariate analysis and an optimization procedure. The validation of the method was conducted using several criteria, including linearity, precision, accuracy, specificity, and robustness. Utilizing Design Expert software for automated QbD method development streamlined the process, leading to a more efficient and robust method compared to traditional manual development techniques. Forced degradation studies, chromatograms of the stressed sample show distinct peaks corresponding to degradation products. The main drug peak remains intact without any significant shift or interference. Degradation peaks are well-separated, ensuring proper resolution. No co-elution of degradation products with the drug peak was observed. The peak purity test confirms that the drug peak is homogeneous and free from degradation interference.

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