RESEARCH PAPER

Quality by Design Approach for the Stability Indicating Method Development and Validation of Selpercatinib Drug Formulation by using RP-HPLC

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ABSTRACT

In method development quality design approach is useful for method goal identification evaluation, method selection and risk assessment. Selpercatinib is a drug used in the treatment of certain types of cancer. The method development includes selecting appropriate chromatographic conditions such as the mobile phase composition, column, and selection wave length. The aim is to achieve optimum separation and quantification of selpercatinib in the dosage form. Optimizing chromatographic conditions by using design expert software. The mobile phase methanol and 0.1% Acetic acid are used. The mobile phase range is 80:20 v/v. The range of this mobile phase is 80:20 .0.7 mL/min found flow rate. The retention time of selpercatinib is 3.20 minutes. The assay was found to be 99.01% for selpercatinib. Optimization was performed. Factorial design performed, i.e., flow rate and percentages of methanol. Analysis of variance confirmed that method parameters. UV detection at 220 nm. The International Council of Harmonization (ICH) guidelines ensure the accurate, precise and reliable results to validate the parameters is an acceptable range. Stress studies reveal that drugs were degraded acidic conditions, alkaline conditions, neutral conditions, oxidative conditions, and photolytic conditions. Hence the proposed method was stability, indicating using quality by design (QbD) approach all the method parameters were better understood. This systematic approach ensures a thorough understanding of the assay, facilitates process optimization, and enables effective control strategies to reduce variability and enhance product quality.

Keywords: Quality by design, RP-HPLC, Selpercatinib, Stability indicating method, Design expert software.

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INTRODUCTION

Critical process parameters and critical quality attributes defining analytical quality by design (AQbD) it help in developing reliable and robust analytical methods. This approach facilitates easier regulatory approval and ongoing quality control of pharmaceutical products.[1-3] Selpercatinib is a highly selective RET kinase inhibitor that targets various RET fusions, activating RET mutations and brain metastases. Its chemical structure consists of a pyrazolo(1,5-a)pyridine core attached to a 6-(2-hydroxy-2-methyl propoxy) and a 4-(6-(((-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo(3.1.1) heptan-3-yl)pyridine-3-yl) group, with a carbonitrile moiety present on the pyrazolo(1,5-a) pyridine ring. The specific kinase inhibit or mentioned is designed to target and inhibit the activity of the rearranged during transfect ion tyrosine kinase receptors with higher selectivity compared to other RTK classes. This selectivity means that the inhibitor has a stronger affinity and ability to inhibit the activity of RET, RTKs while having reduced impact on other RTK. Selpercatinib is indicated to treat non-small cell lung carcinoma and two types of thyroid cancers with alterations in the RET gene together with advanced medullary thyroid cancer.[4]

Structure of selpercatinib shown in Figure 1. The liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[5] bioanalytical method,[6] high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method,[7] reverse-phase high-performance liquid chromatography (RP-HPLC) method has been reported. However, quality by design (QbD) approach stability indicating method were not discussed. Routine analytical work can be

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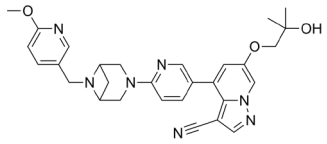

Figure 1: Structure of selpercatinib

streamlined and optimized to minimize the impact of various experimental factors and improve consistency in method performance. Overall, while extensive studies may have their advantages, such as greater control over experimental conditions, they also have limitations in terms of cost, time and generalizing. In contrast, meta-analysis offers a valuable and efficient approach to synthesizing research findings, providing a comprehensive understanding of the combined effects of experimental factors. The developed method was tested and assessed to ensure it meets the criteria and standards set by the International Council of Harmonization (ICH) guidelines for analytical method validation. This involved determining the method's specificity, linearity, accuracy, precision, robustness, and system suitability.[8] Rp-HPLC methods refered for other drugs to study stability parameter and system suitability parameters.[9-14]

MATERIAL AND METHODS

Chemical and Reagents

Selpercatinib 99.36% was procured from Swapnaroop Drugs and Pharmaceuticals, Sambhajinagar.

Hydrochloride acid sodium hydroxide can be obtained from Merck India Pvt Ltd, Which is a subsidiary of Merck KGaA, a leading Global Pharmaceutical and Chemical Company. Purchasing methanol and acetic acid from Fischer scientific.

Apparatus and Equipment

The binary gradient system HPLC is used. UV (DAD) detector quaternary gradient (G130A) pump are used.

The chemstation software is used.

The Wenser ultra sonicators are used. The wenser high precision balance this analytical are used.

The purpose of experimental design and data analysis calculations design expert software are used.

Chromatographic Conditions

Based on the provided information, the stationary phase used in the RP-C-18 Agilent column is Kromasil C18. The dimensions of the column are 100 mm in length and 4.6 mm inner diameter (id). The column is operated at ambient temperature and the selected wavelength is 220 nm. The mobile phase uses methanol and 0.1% acetic acid in an 80:20 v/v ratio. Orthophosphoric acid adjusts the pH is 3.0 of the mobile phase. The injection volume is 20 µL and the flow rate is 0.7 mL/min. The total run time for the analysis is 10 minutes

STANDARD SOLUTIONS PREPARATION

Buffer (0.1% OPA)

The buffer in this concentration of 0.1% orthophosphoric acid (OPA) is being prepared. To prepare the buffer solution, approximately 1-mL solution of orthophosphoric acid solution is added in the 1000 mL volumetric flask. This buffer solution will be used to adjust the pH of the mobile phase is 3. The buffer capacity of the solution will help maintain the pH at a constant value even when small amounts of acid or base are introduced during the experiment.

Standard preparation

- About 10 mg of selpercatinib drug added in the 10 mL of dilutant methanol. This prepares 1000 µg/mL stock solution and then prepares a working standard solution 10 µg/mL.

Optimization of Chromatographic Conditions and Design of Experiments

The optimization process involved varying the composition of the mobile phase, wavelength, and flow rate using the software-provided design of experiments. The responses were recorded to each parameter. Based on the desirability indicated by the software, the optimized chromatographic conditions were selected. These conditions would yield maximum absorbance at a wavelength of 220 nm, using the Kromasil C18 column at ambient temperature. The system suitability parameters, such as peak shape, were considered good for this column. The UV detector was employed for detecting selpercatinib during the elution process. The QbD method allowed for a systematic approach to optimizing the chromatographic conditions, ensuring the desired responses were achieved.

Design Software

Design Expert 10 is a comprehensive and powerful software tool that greatly facilitates the design, analysis, and optimization of experiments in various industries, including manufacturing, pharmaceuticals, chemicals, and more.

Method Validation

Method validation is the process of evaluating the performance characteristics and suitability of an analytical method for its intended purpose. It is necessary to ensure that the method is reliable, accurate, and consistent in generating valid results. The validation process follows guidelines provided by regulatory authorities (ICH). In this case, the method was validated according to the ICH Q2(R1) guideline.[12] During method validation. Several parameters are tested to assess the method's performance. These include:

Linearity

Linearity refers to the relationship between the concentration of a substance and the corresponding response, such as peak area, absorbance, or signal intensity. In this case, a calibration curve was created by measuring the peak areas of selpercatinib at different concentrations. To establish linearity, several points along the concentration range were selected. In this case, 0.1, 0.2, 0.3, 0.4, and 0.5 mL of a working standard solution of selpercatinib were pipetted into 10 mL volumetric flasks and diluted with diluent to make a total volume of 10 mL. The resulting curve can be used to determine the concentration of selpercatinib in future samples by measuring

their corresponding peak areas and applying the calibration curve equation.

Precision

The precision of the method was evaluated by conducting the analysis using three different concentrations (20, 30, and 40 µg/mL) of the pure drug on the same day. This was repeated three times to determine the intraday precision. Additionally, to determine the interday precision, the analysis was performed on three different days over a one-week period. The precision of the method was considered satisfactory as the %RSD was found to be less than 2%. The results, including the mean, standard deviation (SD), and %RSD, were recorded and analyzed.

Percentage recovery

To calculate the percentage recovery, use the following formula:

Percentage Recovery = (Mean area of drug in standard solutions/Mean area of drug in sample) * 100.

Robustness

Robustness refers to the ability of a method or process to remain unaffected by small variations or changes in its parameters or conditions. This means that even with minor variations in the parameters, the method should still provide accurate and consistent results. The evaluation of robustness helps to determine if the method is capable of withstanding normal experimental variations and external factors without compromising the accuracy and precision of the results. In order to assess the robustness, the method parameters are intentionally varied within a certain range. The impact of these variations on the method's performance is then evaluated by analyzing the obtained results. If the method remains reliable and produces consistent and accurate results despite the intentional variations, it can be considered robust. Overall, evaluating the robustness of a method is crucial to ensure its reliability and to determine its suitability for routine analysis or testing. It helps to identify potential sources of variation and optimize the method to minimize their effects, ensuring consistent and accurate results in practical applications.

LoD and LoQ

The detected amount refers to the amount of analyte that is observed or sensed, but it may not be precisely determined or reliable. The LoQ, on the other hand, is the minimum amount of analyte that can be measured with confidence and accuracy. It is the point at which the measurement becomes meaningful and can be trusted for quantification. It is important to note that the LoD and LoQ depend on various factors, such as the sensitivity of the instrument, the detection method employed, the sample matrix, and the analytical procedure itself. These parameters are typically determined through the analysis of blank samples with progressively lower concentrations of the analyte of interest. In summary, the LoD represents the detection limit of an analyte, while the LoQ represents the quantitation limit. Both are crucial parameters used in analytical chemistry to assess the sensitivity and reliability of an analytical procedure.

Assay

The assay for selpercatinib was performed by taking 20 capsules and grinding them finely. The purity of selpercatinib was then reported as a percentage. To prepare the solutions for assay, concentrations of 30 parts per million (ppm) were made from both the formulation and a standard. These solutions were then injected into the instrument to record the corresponding areas. The recorded areas from the solutions were used to determine the purity of selpercatinib. This information is crucial for quality control purposes and ensuring that the medication is manufactured within the desired specifications.

Force segradation study

- *Acid degradation*

In 0.1 N HCL added 0.3 mL sample from stock API and then again add 5 mL of 0.1 N HCL and make up volume with diluting of methanol. Take HPLC reading after 1, 2 hours before injecting neutral sample.

- *Alkali degradation*

About 0.1N NaOH added 0.3 mL of sample stock API and then again add 5 mL of 0.1N HCL and made up the volume with a dilutant of methanol. Take HPLC reading after 1, 2 hours before injecting neutral sample.

- *Oxidative degradation*

3%H_2O_2, then add sample from stock API then again add 5 mL of 0.1N HCL and make up volume with dilutant of methanol. Take HPLC reading after 1 or 2 hours before injecting neutral sample.

- *Neutral degradation*

Take 0.3 mL sample from stock API add 5 mL water and make up the volume with dilutant of methanol. Take HPLC reading after 1, 2 hours.

- *Photolytic degradation*

The HPLC system was used to analyze the samples taken at various time points after the 50 mg of selpercatinib was uniformly spread in a petri dish and exposed to direct sunlight for 8 hours.

RESULT AND DISCUSSION

Defining ATP

Implementation helps in demonstrating the reliability, robustness, and suitability of the analytical method. ATP stands for analytical target profile. It is a set of performance requirements and specifications that an analytical method should meet in order to ensure the quality of the final product. The QTPP defines the desired characteristics of the final product, and the CQAs are the attributes that are critical for ensuring the quality of the product. The ATP is then developed to address these CQAs. In the process of ATP development,

various aspects of method development are considered. This includes the selection of the mobile phase, which determines the separation of analytes and their retention on the chromatographic column. The instrument requirements are defined to ensure that the method can be performed accurately and reliably using the available equipment.

Sampling and sample characteristics are also considered in the ATP development. This includes determining the appropriate sample size, representative sampling, and handling of the sample matrix. Standard and sample preparation methods are defined to ensure accurate and reproducible results. Solubility and pH of the analyte are important considerations in method development. These factors affect the stability and detection efficiency of the analyte.

Selection of Detection Wavelength

A standard solution of selpercatinib drug with a concentration of 30 µg/mL was used for the measurement. The UV range of 200 to 400 nm was scanned and the absorbance wavelength of 220 nm was selected. Figure 2 shows selection of wavelength.

Optimization of Proposed Method

ANOVA study

This optimization process involves adjusting the levels of the predictor variables in order to maximize or minimize the response variable, depending on the specific objective. To optimize the proposed method for retention time, the first step would be to define the desired target value for retention time. This could be a specific value that meets the requirements of the experiment or a range within which the retention time should fall. Next, the various experimental factors that can affect retention time would be identified. These factors could include temperature, pressure, flow rate, column length, solvent composition, and any other relevant variables. Once the factors are identified, they can be systematically adjusted within their respective ranges to find the optimal combination that gives the desired retention time. This can be done using methods like response surface methodology or design of experiments. Responses by changes in flow rate, solvent are shown in Table 1.

During the optimization process, the response variable (retention time) would be measured for each combination of factor levels. The data obtained would then be used to fit a regression model, such as a quadratic model, to describe the relationship between the factors and the response. The fitted model can then be used to find the factor levels that maximize or minimize the response variable. This can be done by solving the quadratic equation or by using numerical optimization techniques.[13] Once the optimal factor levels are determined, confirmation experiments can be conducted to validate the results and ensure that the desired retention time is achieved consistently. Overall, the optimization of the proposed method involves using ANOVA analysis to compare the quality of different models, fitting a quadratic regression model to describe the relationship between factors and the response variable, and systematically adjusting the factor levels to find the optimal combination that gives the desired retention time.[14] Figure 3A shows Normal Plot of Residuals for Retention time, Plot of Residuals Vs Run for Retention time and Plot of Predicted vs. Actual data for Retention time, also figure 3B shows 2 D Contour plots of retention time as a methanol and flow rate & 3 D Contour plots of retention time as a methanol and flow rate respectively.

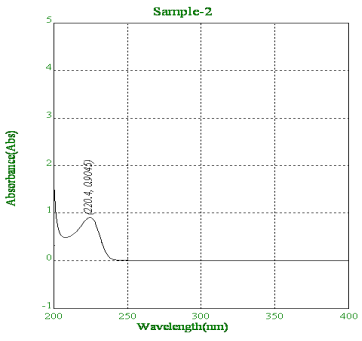

Figure 2: Selection of wavelength

Method Validation

Linearity

Based on the observations from linearity studies and the calibration curve with an r2 value of 0.999, a linear relationship

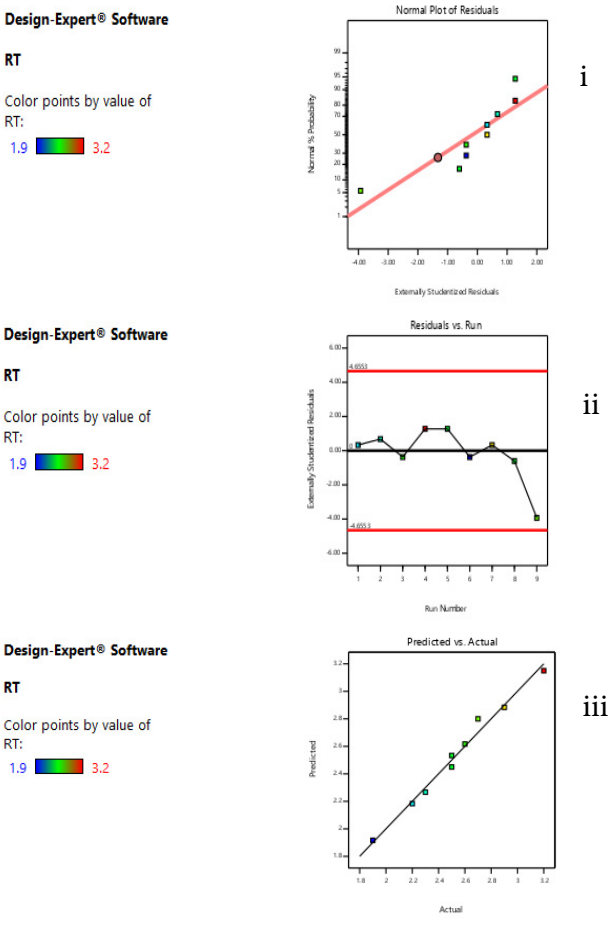

Figure 3: (i) Normal plot of residuals for retention time, (ii) Plot of residuals vs run for retention time. (iii) Plot of predicted vs. actual data for retention time, respectively.

Table 1: ANOVA for linear model

Source	*Sum of squares*	*Df*	*Mean square*	*f- value*	*p-value*
Model	1.16	2	0.5808	190.09	0.0001>
A-Flow rate	0.7350	1	0.7350	240.55	0.0001>
B- Methanol	0.4267	1	0.4267	139.64	0.0001>
Residual	0.0183	6	0.0031		

Table 2: Responese: RT

		Factor 1	*Factor 2*	*Resopnce E1*	*Resopnce E2*
Std	*Run*	*Flow rate*	*B – methanol*	*RT-*	*Area*
		ML/min	*%*	*Min*	
6	1	0.8	75	3.4	2089.58
2	2	0.7	75	3.9	2297.41
1	3	0.7	85	3.5	2560.96
7	4	0.8	80	3.2	2138.26
9	5	0.9	85	2.7	1963.66
3	6	0.8	85	3.08	2168.91
4	7	0.7	80	3.6	2407.81
5	8	0.9	75	3.1	1922.23
8	9	0.9	80	2.8	1949.13

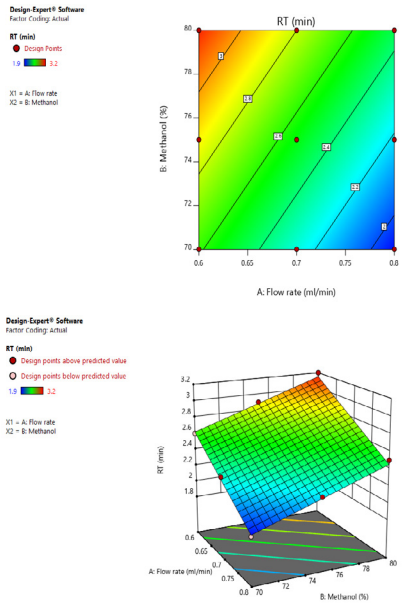

Figure 4: 2D Contour plots of retention time as a methanol and flow rate; 3D contour plots of retention time as a methanol and flow rate, respectively

was established between drug concentration and area. The equation of the line, y=mx+c, shows the slope (m) and y-intercept (c). Furthermore, a linear correlation between drug concentration and area was found within the range of 10 to 50 µg/mL. This indicates that this method can be effectively used for drug concentration determination within this concentration range. Linearity of selpercatinib shown in Figure 4.

Precision

This information suggests that the method used was able to consistently produce accurate and reliable results. The

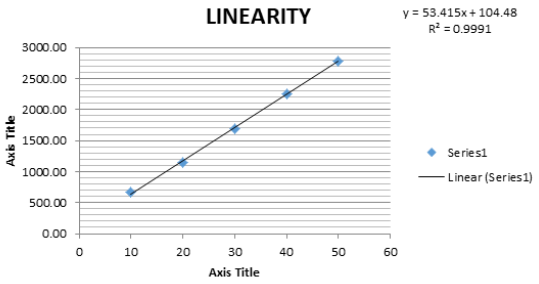

Figure 4: Linearity of selpercatinib

Table 3: Intraday and interday precision

Intraday precision			*Interday precision*		
Concentration	*Mean area*	*%RSD*	*Concentrations*	*Mean area*	*%RSD*
20	1145.81	0.00	20	1146.68	0.12
30	1688.74	0.10	30	1689.23	0.11
40	2253.64	0.06	40	2257.07	0.16

Table 4: Accuracy results

S No.	*Concentration*	*Area*	*SD*	*% RSD*
01	10	1071.00	966	0.074
02	20	1158.96	1054	0.110
03	30	1273.43	1169	0.069

%RSD values were within the acceptable limits for precision, indicating that the readings obtained were not significantly varied and thus precise. The mean area and standard deviations also support the precision of the method. Overall, these results provide evidence that the method can be considered precise for the purpose of the study or analysis. Intraday and Interday precision shown in Table 2B.

Accuracy

The test was successfully passed, meeting the specifications of having a relative standard deviation (RSD) less than 2. Accuracy results are shown in Table 2C.

%Recovery

The recovery of selpercatinib was within the acceptable limit. Recovery studies shown in Table 2D.

Robustness

This suggests that the method used in the study is robust, meaning that small changes do not easily influence it in the experimental conditions. The %RSD (relative standard deviation) values being less than two indicate that the results obtained were consistent and reproducible. Therefore, researchers can have confidence in the reliability and consistency of the method. Result of Robustness Studies by change in wavelength (nm) and flow rate shown in Table 2E.

LoD and LoQ

LoD = 3.3 * SD / S

LoQ = 10 * SD / S

The LoD was found to be 3.28 and LoQ was 9.95 µg/mL

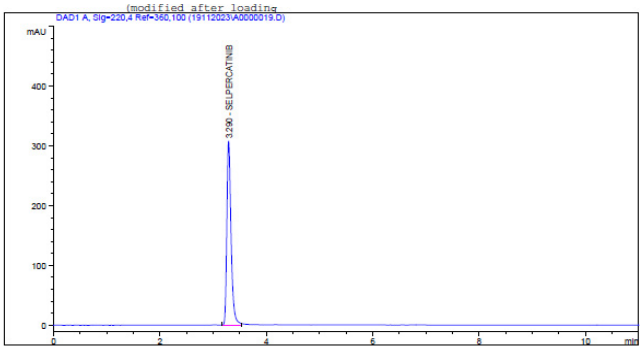

Figure 5: Assay of selpercatinib

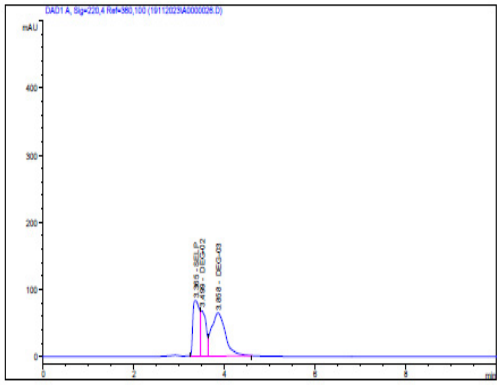

Figure 5B: Chromatogram of acid degradation

Assay

Assay results are shown in table 2 F and Assay results are shown in Figure 5A.

Assay results are shown in Table 8.

The result obtaining an acceptable range

Table 5: Recovery studies

Recovery level (%)	*Concentration of test (µg/mL)*	*Concentration of standard added (µg/mL)*	*Amount found (µg/mL)*	*%Recovery*
50	20	20	25	99.00
100	20	25	30	99.50
150	20	40	35	99.51

Table 6: Result of robustness studies wavelength (nm)

S. No .	*Parameter varied wavelength (nm)*	*Concentration (µg/mL)*	*Area*	*%RSD*
01	248	20	2723.70	0.10
02	250	20	2824.39	0.82

Table 7: Flow rate

S. No.	*Parameter varied flow rate*	*Concentration (µg/mL)*	*Area*	*%RSD*
01	0.8	20	3043.88	0.10
02	0.9	20	2448.07	0.15

Table 8: Assay of selpercatinib

S. No.	*Concentration (µg/mL)*	*Area of standard*	*%RSD*	*%Assay*
01	30% Assay	1690.82	0.187	99.01%

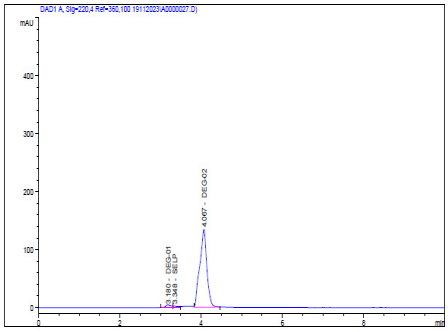

Figure 5C: Chromatogram of alkali degradation

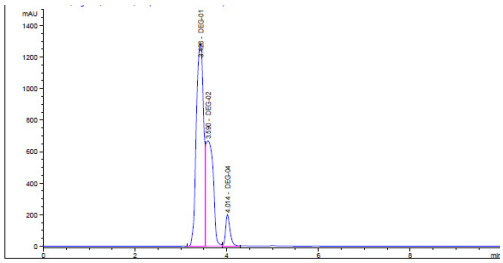

Figure 5D: Chromatogram of oxidative degradation

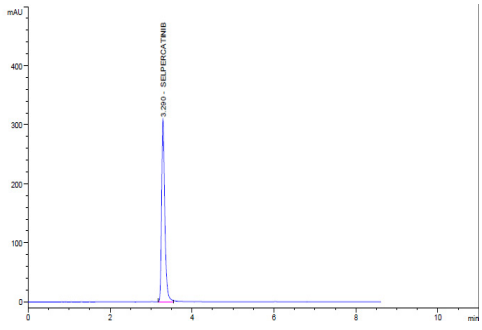

Figure 5E: Chromatogram of neutral degradation

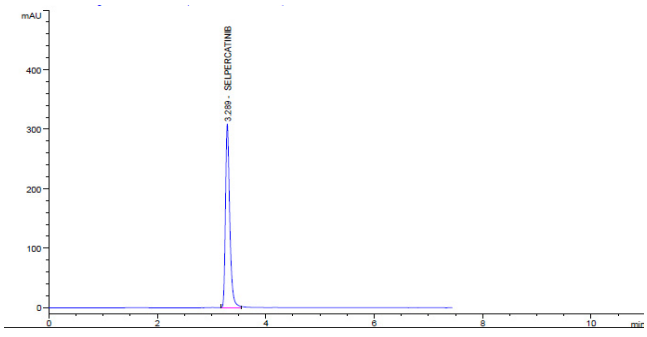

Figure 5F: Chromatogram of photolytic degradation

Table 9: Percent degradation

S. No.	*Degradation study*	*%Degradation*
1	Acid degradation	49.01
2	Basic degradation	98.71
3	H_2O_2 degradation	100
4	Neutral degradation	1.82
5	Photodegradation	2.66

Force degradation studies

The forced degradation studies of selpercatinib suggest that the drug is stable under thermal and photolytic stress conditions. However, it degrades in alkaline conditions and is susceptible to more oxidative stress then basic stress condition and acidic conditions. This information is important for determining appropriate storage and handling conditions for the drug, as well as understanding its potential degradation pathways. Further investigations may be needed to identify the specific degradation products and assess their potential impact on the drug's efficacy and safety. Force degradation studies by acid degradation, alkali degradation, oxidative degradation, and neutral solution, and photolytic degradation shown in figure 5B-5G. Table 2G represents percent degradation.

CONCLUSION

The developed method analysis of selpercatinib in pharmaceutical dosage forms using a systematic QbD approach has been found to be simple, sensitive, robust, and cost-effective. The validation results showed good linearity, accuracy, precision, robustness, and specificity, confirming the reliability of the method. Furthermore, degradation studies in accordance with the ICH guidelines confirmed that the degradation of selpercatinib could be easily detected by characteristic peaks. This information is crucial for assessing the stability and shelf-life of the drug. The use of QbD in the development of this analytical method provides several advantages over traditional methods. It allows for a more systematic and efficient approach, reduces the risk of method failures during transfer, and enhances the overall understanding of the method's performance. The developed QbD-based RPHPLC method is a valuable tool for the analysis of selpercatinib. Its simplicity, sensitivity, robustness, and cost-effectiveness make it suitable for routine quality control and stability testing.

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