Int. J. Pharm. Investigation, 2026; 16(1):201-209.
https://www.jpionline.org

Original Article

New RP-HPLC Method Development and Validation, Incorporating Quality by Design (QbD) with Force Degradation Study for Alectinib Estimation: A Green Approach

Jyoti Tangde*, Sanjay Sawant

Department of Pharmaceutical Chemistry, Smt. Kashibai Navale College of Pharmacy (Affiliated to Savitribai Phule Pune University, Pune), Kondhwa, Maharashtra, INDIA.

ABSTRACT

Objectives: The present research work focuses on development and validation of Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) for the estimation of alectinib with force degradation study based on Quality by Design (QbD) analysis. **Materials and Methods:** Two-level optimal factorial design has been used for method optimization, using design expert software. The C_{18} column was used for chromatographic separations at a 265 nm wavelength, 1.00 mL min^{-1} flow rate, and the run time was 10 min at ambient temperature. Risk assessment and statistical analysis were performed to demonstrate the significance of design. The assessment of greenness of this study was performed using the AGREE tool. **Results:** The validation was carried out using ICH Q2R1 guidelines and results found linearity R^2=0.999. Retention Time (RT) was observed at 3.212, the Limit of Detection (LOD), and Limit of Quantitation (LOQ) were found to be 0.3861 µg/mL and 1.1701 µg/mL, respectively. The Relative Standard Deviation (RSD) for intra-day and inert-day precision was found to be 1.5652 and 1.730 respectively. The forced degradation studies results showed alectinib susceptible to acidic, thermal, and photolytic conditions. No degradation was found in alkaline conditions. **Conclusion:** The resulting method with QbD and Green approach holds potential application in routine analysis of alectinib.

Keywords: Alectinib, Degradation, HPLC, Optimal Design, Method Greenness.

Correspondence:
Ms. Jyoti Tangde
Department of Pharmaceutical Chemistry, Smt. Kashibai Navale College of Pharmacy (Affiliated to Savitribai Phule Pune University, Pune), Kondhwa, Maharashtra, INDIA.
Email: jyotitangde@gmail.com

Received: 09-04-2025;
Revised: 24-06-2025;
Accepted: 18-08-2025.

INTRODUCTION

The structure of alectinib consists of a tetracyclic hetero ring system with 6,6-dimethyl-5,6-dihydro-11H-benzo[b]carbazol-11-one having additional cyano, 4-(morpholin-4-yl) piperidin-1-yl, and ethyl substituents (NCBI, 2024). It is a newly developed Anaplastic Lymphoma Kinase (ALK) inhibitor used in the treatment of malignant and Non-Small Cell Lung Cancer (NSCLC). Inhibition of ALK leads to prevention of downstream signaling and cell proliferation and slows down the tumour continuation (Beardslee and Lawson, 2018). This drug was approved by the USFDA on 6th November 2017 for treatment of NSCLC. (National Cancer Institute Report, 2017) It is a second-generation ALK inhibitor found to be superior to Crizotinib (a first-generation ALK inhibitor). First-line treatment of alectinib manifests a 65-72% reduction in the rapid death or progression rate of NSCLC as compared to crizotinib (Samacá-Samacá *et al*., 2023).

The Quality by Design (QbD) approach was used for the method optimization and stability study of alectinib. QbD is a comprehensive process design, development, and manufacturing processes with presumed product and process specifications. QbD is a scientifically designed approach to meet specific objectives. It provides thorough knowledge to create chromatographic databases that can be employed to produce other methods if needed in the future. The QbD is specified in ICH Q8 guidelines, which affirm "the quality cannot be tested in products; quality should be built in design"(Patel *et al*. 2021; ICH Q2R1Guidelines) Overall, the AQbD technique detects early assessments of risk and resolves potential concerns, making the established method more trustworthy; hence, current work focuses on the application of QbD for the development of a novel method for alectinib quantification (Meshram and Ranpise, 2024).

The analytical studies reported earlier for method development and validation using liquid chromatography for alectinib include RP-HPLC (ICH Q14 2022; Prashanthi *et al*., 2018). HPLC-PDA

DOI: 10.5530/ijpi.20260313

for determination of alectinib concentrations in the plasma (Pavani *et al*., 2019), HPLC-MS/MS with simultaneous estimation of alectinib, crizotinib, erlotinib, gefitinib, and osimertinib (Lee *et al*., 2020). Besides a few methods reported for HPLC development, only two methods are reported for stability study. The simultaneous estimation with a stability study of brigatinib and alectinib (Veelen *et al*., 2021) and the UPLC-PDA forced degradation study of alectinib (Gunturu and Kantipudi, 2022). Since no QbD-based optimization and stability study of alectinib has been conducted previously, the current study focuses on method development and validation, as well as an alectinib stability study using the QbD methodology.

MATERIALS AND METHODS

Materials

Alectinib (purity 99.06%), a yellow, white, crystalline solid. Alecensa capsules containing 150 mg of alectinib in each capsule, manufactured by Roche Product India Pvt. Ltd., Methanol, methanol of HPLC grade obtained from Merck Life Sciences, Mumbai, Triethylamine and Orthophosphoric acid AR grade obtained from Research Fine Chemical Industries, Mumbai.

Instrumentation

The chromatographic separations were performed with isocratic mode of separation using waters HPLC. The C18 analytical column was used having dimension 4.6×250 mm with 5 µm particle size. UV detection was performed at 265 nm with 10 µL injection volume, 1.00 mL min^{-1} flow rate, at ambient temperature for 10 min run time.

Methods

Preparation of primary stock solution

The 10 mg of alectinib was weighed accurately and dissolved into 10 mL of methanol to get 1000 µg/mL. From this, 0.1 mL was transferred in 10 mL of methanol to get 10 µg/mL.

Preparation of working sample

The working solution was prepared for a series of concentrations from 0.5 to 50 µg/mL of alectinib using the primary stock solution and was filtered using a 0.45 µm filter prior to injecting chromatographic analysis.

Quality Target Product Profile (QTPP)

The QbD approach begins with the quality target product profile identification, based on literature surveys and previous studies, Alecensa capsules was identified as QTPP (Manohar *et al*., 2022).

Critical Quality Attributes (CQA) and Critical Process Parameters (CPP)

To attain the considered output, i.e., an accurate, specific, and robust analytical method, to meet the ATP criteria, CQAs, and CPPs were selected. These factors were selected based on the ishikawa fishbone diagram, which influences the analytical method development. The alectinib retention time, asymmetry factor, and theoretical plates were chosen as CQAs for method optimization, whereas peak area and retention time were chosen as CQAs for stability testing. The pH of the mobile phase and concentration of the methanol contained in the mobile phase were chosen as CPP for method optimization, while storage conditions and sampling days were considered as CPP for the stability study, which could have a substantial impact on the method's performance (Jadhav *et al*., 2014; Durga *et al*., 2022; Vora and Shah, 2019).

RESULTS

Experimental design (Optimal factorial design)

Optimal design is an alternative to general factorial design, which produces all possible combinations and runs that are willing to perform. Based on the specified model with 2 factors, eliminated those interactions from the model to reduce the number of runs and perform all feasible generated runs (Frederick and Alireza, 2011; Ramalingam *et al*.,2015). Two level optimal factorial designs were used, and the software suggested 9 runs, as shown in Table 1.

The independent factors were selected as the composition of the mobile phase, which facilitates the adding of methanol from 70 to 90% v/v. Moreover, the pH of the aqueous phase was considered from 5 to 8. The selection of concentration of organic phase and pH was decided from the structure of compound (Harish *et al*., 2023).

The dependent factors were selected as retention time, theoretical plates, and asymmetric factor. After the selection of dependent and independent factors, trials were performed, and optimization was executed depending on the desirability function. The optimization finds a point which accelerates the desirable functions, and this value completely depends on the closeness of upper and lower limits to the current optimum (Sharma *et al*., 2023; ICH. Q2R1 Guideline, 2005).

Optimized chromatographic conditions

The mobile phase consisted of a combination of methanol and water in ration 90:10 v/v with a pH 8. The pH was adjusted using phosphoric acid and triethylamine. The detection wavelength was set at 265 nm to obtain an optimal response. The retention time was achieved at 3.212 min, the asymmetric factor was 1.357, and theoretical plates were obtained at 9899.

Desirability value

The desirability value (i) ranges between 0 and 1, i= 0 indicates an undesirable response and i =1 indicates the complete desired response. Therefore, the following trials with maximum

desirability (i=0.967) as shown in Figure 1a, were selected for method optimization (Gamel *et al*., 2021).

The Effect of independent variables on process variables

(Figure 1: 3D plot a) shows antagonistic effect with change in buffer's pH, and retention time decreases with increase in quantity of methanol. 3D plot b) represents an antagonistic effect of pH on asymmetric factor, and synergistic effect with amount of methanol. Plot c) represents increase in pH, decreases the number of theoretical plates and increase in quantity of methanol increases the theoretical plates.

Statistical analysis

The effect of independent variables on process variables

The equation for response on retention time (X), assymetric factor (Y), theoretical plates (Z): 2FI model response is given below.

Retention time (X)=+3.21200-1.08 * A -0.027 * B -0.079 * A * B

Assymetric factor (Y)=+1.48+0.018* A-0.13* B-0.010*A * B

Theoretical plate (Z)=+8169.46+1706.79* A-293.96* B+57.71* A * B

The + sign indicates increasing, and the - sign indicates decreasing the concentration of individual factors. Factor A (change in mobile phase composition) while B (pH). So, a change in the concentration of the organic phase (methanol) can change the X, Y, and Z. The overall effect of the above equations shows a slight change in the concentration of methanol, changing the retention time and asymmetry, while the concentration of the mobile phase and pH have a significant effect on the theoretical plate (Z) of alectinib.

Statistical analysis (ANOVA)

After applying experimental design, model terms for retention time, asymmetric factor and theoretical plates, p value < 0.005 and "Prob > F" indicate model significance. The summary of ANOVA for retention time, asymmetric factor and theoretical plates is given in Table 2.

Development of Method

The optimized chromatographic conditions show very good separation of alectinib in less time. Retention time was obtained at 3.212 min. (Figure 2) with system suitability parameter in Table 3.

Method Validation

Validation was carried out using Q2 (R1) ICH guidelines with respect to System Suitability Studies (SSS), linearity, LOD, LOQ, accuracy, precision and robustness (Gamel *et al*., 2021; Chowdary *et al*., 2020) the summary of validation is given in Table 4.

Calibration Curve (Linearity)

Ten mg of alectinib was dissolved in 10 mL of methanol. The mobile phase was employed to do additional dilutions of 5 to 30 µg/mL. Furthermore, the final solutions were subjected to a 5 min ultrasound. After drawing the calibration curve and determining the linearity (range) of 5 µg/mL to 30 µg/mL, R^2=0.9999 was identified.

Selectivity

For performing selectivity, Alecensa 150 mg capsules were used. Ten (10) capsules of alectinib without shells were triturated, and 14.23 mg (10 mg of API) with excipients was added in to a volumetric flask (10 mL), volume using methanol. Chromatographic parameters of formulation were collated with the API. System suitability parameters were found. Retention time=3.212 min, peak area=965421, tailing factor=1.357, and theoretical plates=9640, respectively. Results conclude that excipients of capsules do not show interference in separation of drug in optimized mobile phase (Nhavale *et al*., 2024).

Detection Limit (LOD) and Quantitation Limit (LOQ)

Sensitivity of these methods was determined by calculating the LOD and LOQ using the below formula. Injecting the six concentrations, i.e., 5 to 30 ug/mL, and considering the slope and standard deviation of the calibration curve, the calculated LOD and LOQ were found to be 0.3861 µg/mL and 1.1701 µg/mL, respectively.

$$LOD=3.3 * \sigma / S$$

$$LOQ=10 * \sigma / S$$

Where,

σ=standard deviation of the response.

S=slope of the curve.

Accuracy

Accuracy was performed using three concentrations (n=3) it was resolved at three different levels: 80,100 and 120% of drug and accuracy results were found between specified ranges of 99.92 to 100.36%. The concentrations with % recovery results are depicted in Table 4.

Precision

Intraday and inter-day precision studies were conducted using 6 replicates of 20 µg/mL solution in a day. The RSD for intraday was found to be 1.6193. Moreover, the interday precision of samples was performed for 3 days and RSD was found to be 1.789. Repeatability was carried out by preparing 6 concentrations

and RSD was found at 0.566. The results of precision show the obtained results are reliable with the ICH guidelines.

Robustness

The robustness of this method was tested using a 10 µg/mL solution and at three levels purposefully changed experimental conditions. The change in mobile phase composition (89-91v/v), pH variations (7.8-8.2), wavelength variation (263-267 nm), and flow rate variation (0.9-1.1 mL/min) were the parameters. The approach is robust, as evidenced by chromatograms.

Stability study of alectinib

Hydrolysis (Acid degradation studies)

The 1 mL of alectinib was taken from the stock solution and 1 mL of 1N HCl was added in to it, then it was refluxed for 30 min at 40°C/60°C for 1 to 5 sampling days. The 50 µg/mL solution was prepared from the resulting solution and 0.30 µL of this was injected into the system to record the chromatograms (shown in Figure 3) to determine the stability of the sample.

Results of hydrolysis by 1 N HCl

The percentage degradation of alectinib was found to be 13.32% in acidic condition for day 1 and 29.95% for day 5 at 60°C; whereas 11.23% for day 1 and 18.59% for day 5 was found at 40°C. The extra peaks were eluted at retention time 6.359 min.

Alkali Degradation

From stock solution, 1 mL of alectinib was added along with 1 mL of 1 N NaOH, and mixture was refluxed for 30 min. at 60°C. Prepared 50 µg/mL solution and 0.30 µL was injected into the HPLC system to record chromatograms.

Results of alkali degradation by 1 N NaOH

No degradation of alectinib was observed in alkaline condition at 40°C and 60°C for day 1 and day 5 as well. As alectinib contains four nitrogen atoms in its structure, basicity of drug is high and hence, when exposed to alkaline condition, no degradation was seen. As the nature of both API and NaOH is same, the extra peaks were not eluted in given run time and the chromatogram is depicted in Figure 3.

Peroxide degradation studies

From the stock solution of alectinib, 1 mL was taken in 1 mL of 20% hydrogen peroxide (H_2O_2) and maintained at 25°C and 60°C for 30 min. The resulting solution was diluted to create a 50 µg/mL solution and 0.30 µL was injected into the HPLC system. Chromatograms were recorded to determine sample stability. One prominent degradant peak was observed.

Results of oxidation by 20% H_2O_2

The percentage degradation of alectinib in oxidative condition was found to be 44.936% for day 1 and 56.681% for day 5 at 25°C; whereas 49.572% for day 1 and 69.367% for day 5 at 60°C. The percentage degradation was not within acceptable criteria (NMT 10%). One extra peak was eluted at 7.364 min in a given run time and the chromatogram shown in Figure 3.

Thermal degradation studies

For thermal degradation studies of alectinib sample was retained in an oven at 30°C to 105°C for 6 hr to investigate thermal degradation from day 1 to 5. HPLC analysis was carried out by diluting the resulting solution to 50 µg/mL solution, injecting

Table 1: Runs and their results for optimized mobile phase.

Sl. No.	Composition of (Organic Phase)	Buffer pH	Retention Time (RT)	Asymmetric Factor	Theoretical Plates (N)
1	90	8	3.211	1.367	9381
2	90	8	3.212	1.357	9899
3	70	5	6.234	1.569	6528
4	70	5	6.231	1.524	6374
5	70	5	6.219	1.627	7541
6	70	8	6.392	1.328	6524
7	90	5	3.298	1.624	10264
8	90	5	3.267	1.634	9961
9	70	8	6.267	1.357	5698

Table 2: Summary of ANOVA.

Sl. No.	Model terms	*p* value	F value	R^2	Pred. R^2	Adj. R^2
1.	Retention time	0.0001	4018.33	0.9996	0.9984	0.9993
2.	Asymmetric factor	0.0001	39.36	0.9594	0.9023	0.9350
3.	Theoretical plates	0.0001	32.27	0.9509	0.8857	0.9214

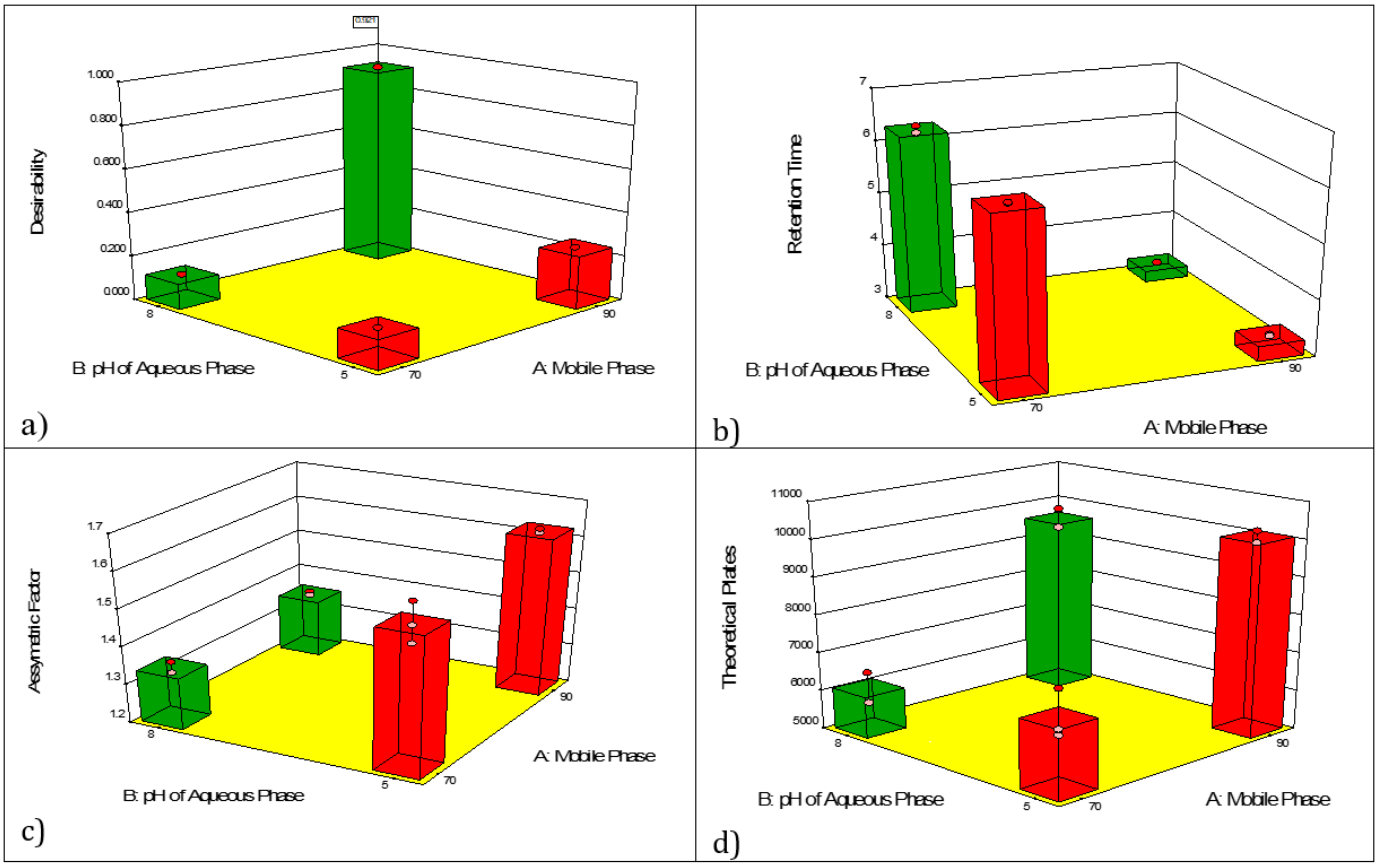

Figure 1: 3D plots for a)Desirability function, b) retention time, c) asymmetric factor, and d) theoretical plates with effect of mobile phase composition and pH.

0.30 µL solution into HPLC system to record the chromatograms for determination of stability.

Results of thermolysis

The % degradation of alectinib was found to be 5.67% in thermolytic condition for day 1 and 31.95% for day 5 at 30°C and 39.68% for day 1, 58.49% for day 5 at 105°C One extra peaks were eluted on 9.647 min the chromatogram was shown in Figure 3.

Photolytic degradation studies

The photolytic stability of alectinib was investigated by exposing the solution of alectinib to UV light for three days in a UV chamber (200 Watt/hr.) in a photochemical chamber. Then it was diluted to prepare a 50 µg/mL solution, and 0.30 µL of this solution into the HPLC system to record the chromatogram. Major degradation was observed in photolytic conditions.

Results of Photolysis

The percentage degradation of alectinib was found to be 18.95% in photolytic conditions for day 1 and 68.64% for day 5 by Light 1x ICH; while it was 39.99% for day 1 and 83.64% for day 5 by Light 3x ICH. The extra peaks were eluted at retention times of 12.441 min and the chromatogram is shown in Figure 3a for 1xICH for day 1 and 3b 3xICH for day 5.

Table 3: System suitability Parameter.

Sl. No.	Parameter	Observations
1.	Retention Time	3.212 min
2.	Peak Area	965421
3.	Asymmetric Factor	1.357
4.	Theoretical plates	9899

The greenness assessment of the above method was performed using the AGREE tool, which offers reliable and precise findings for method greenness. Following the input of all 12 green chemistry principles into software on a 0 to 1 scale, which is indicated by a red, yellow, or green colour, the graph is generated automatically by the software. The entire result is displayed centrally, i.e., 0.74, with a value closer to one and a darker green signifying how much greener and more eco-friendly the process is (Figure 4). The estimated score of 0.72 indicates that the analyzed method is more ecologically friendly (Bairagi *et al.*, 2024; Koradia *et al.*, 2024).

DISCUSSION

As per the literature and earlier studies performed for HPLC analysis of alectinib, none of the studies demonstrate QbD implementation for method development and stability studies of alectinib with predefined design variables. In this context, a new

method using the QbD approach with stress degradation studies of alectinib was performed as per ICH Q2R1 guidelines.

Optimization

The optimization was performed using a two-level optimal design, and software suggested 9 runs. The independent factors were selected as the composition of the mobile phase and pH of the aqueous phase, mobile phase composition with the adding of methanol was consisted of 70-90% v/v and pH was selected from 5 to 8. The selection of concentration of organic phase and pH was decided from the structure and pKa of alectinib.

The effect of independent variables demonstrates the antagonistic effect of pH and the composition of the mobile phase on retention time. For asymmetry, an antagonistic effect was seen with pH and a synergistic effect with the amount of methanol. For several theoretical plates, there is an antagonistic effect with pH and a synergistic effect with the amount of methanol.

Validation

The validation of the above method was performed according to ICH Q2R1 guidelines, and the results show linearity was obtained with an R2 value of 0.9999, and recovery was achieved between 99.92% and 100.36%. The relative standard deviation for intra-day and inter-day was achieved at 1.693%. and 1.759%, respectively. The LOD and LOQ were obtained as 0.3861 µg/mL and 1.1701 µg/mL. A specificity study was conducted using the Alecensa 50 mg capsule, indicating no interference of the excipient. Robustness of these methods which performed using the four parameters i.e. change in Mobile phase concentration, pH, detection wavelengths, and flow rate found out with RSD less than 2. So, the present RP-HPLC method was found to be linear, sensitive, precise, specific, and robust.

Stability study

A stability study was performed at specified temperature and storage conditions. Results of stress degradation studies show alectinib is susceptible to acid, oxidative, thermal, and photolytic degradation. It was found to be stable in alkaline conditions as the basicity of the drug is high, and when it reacts with alkali like NaOH, it doesn't find any degradation, summary of degradation of alectinib shown in Table 5.

Table 4: Validation parameters summary.

Sl. No.	Specifications	Validation Results
1.	Linearity (*n*=6)	5-30 µg/mL
2.	Regression Equation	y=193370x +13333
3.	Correlation coefficient (R^2)	0.9999
4.	Accuracy (*n*=3) 80% 100% 120%	% Recovery 99.92±0.40 99.95±0.41 100.36±0.40
5.	Intra-day Precision (*n*=3) 20 µg/mL Inter-day Precision (*n*=3) 20 µg/mL	Mean±RSD (%) 1.6193±0.47 1.789±0.44
6.	LOD (20 µg/mL) (*n*=6)	0.3861 µg/mL
7.	LOQ (20 µg/mL) (*n*=6)	1.1701 µg/mL
8.	Robustness (*n*=3) Mobile phase concentration (v/v) Wavelength (nm) Flow rate (mL/min) Buffer pH	Mean±RSD (%) 2.491±1.40 0.982±0.54 2.381±0.44 1.1371±0.56
Standard Peak Area of Alectinib: 30 ug/mL: 5993346		

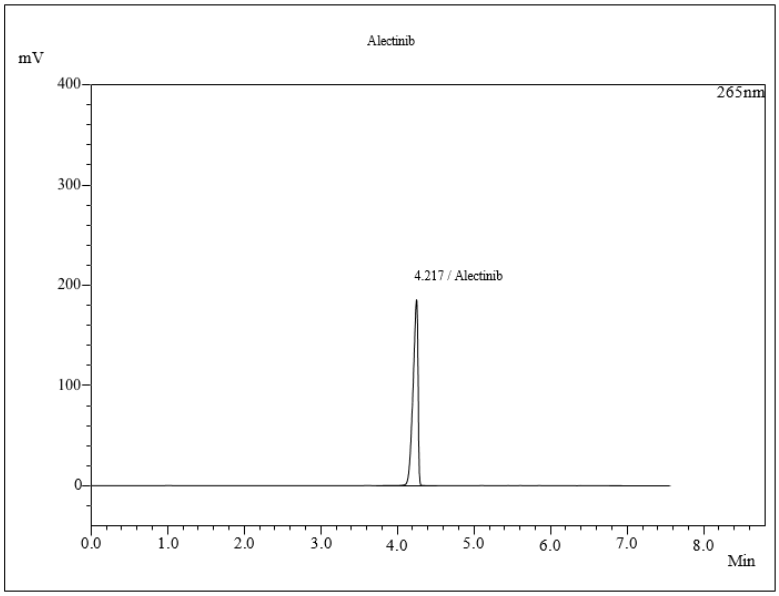

Figure 2: Optimized Chromatogram for Alectinib (30 ug/mL).

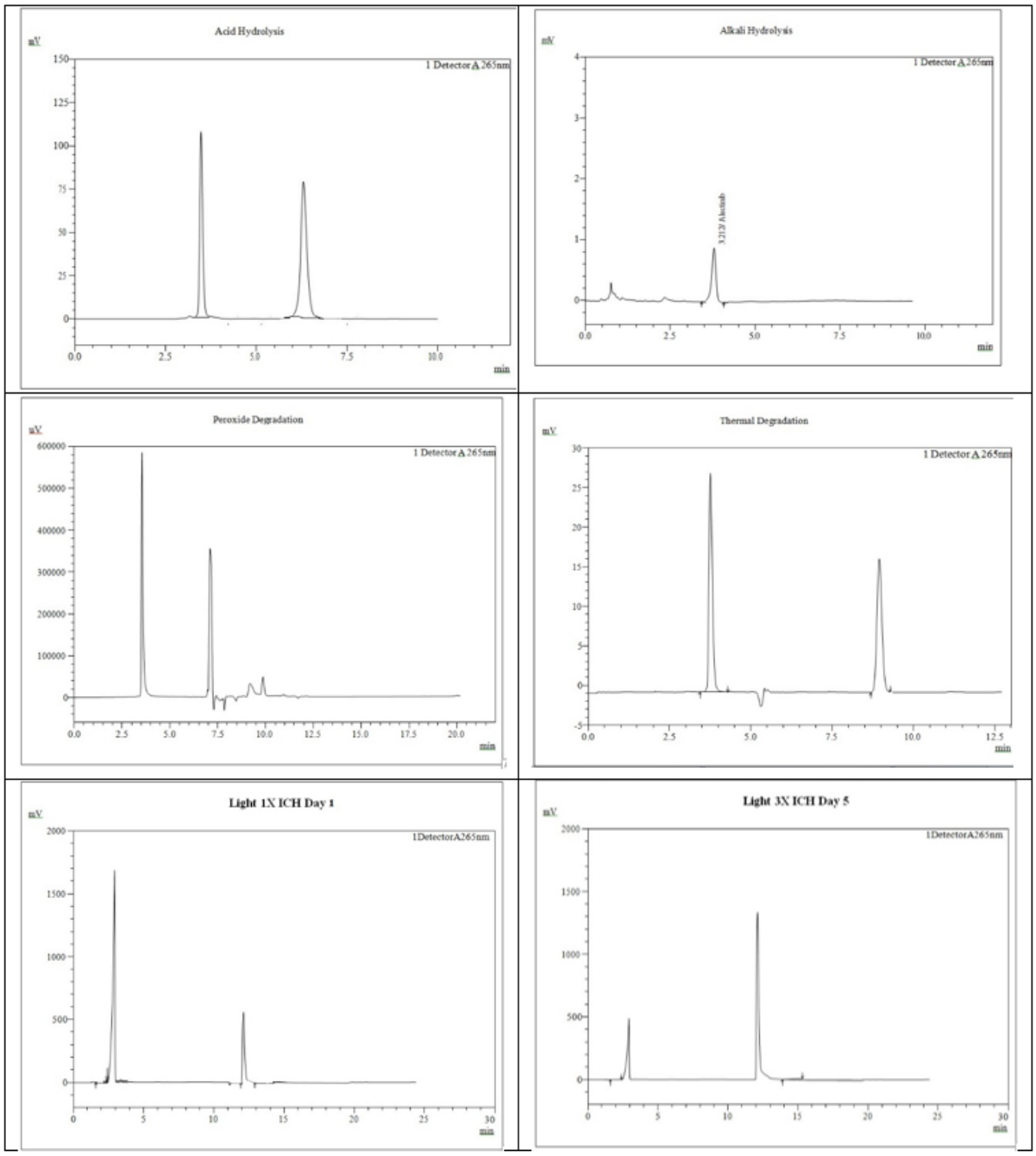

Figure 3: Chromatogram for acid, alkali, peroxide, thermal and photolytic degradation.

Acid Hydrolysis

Stability studies are performed to ensure the standard of a drug product and its effectiveness all through its shelf life. The acid hydrolysis for the above method software suggested conditions were experimentally conducted, and results found out Degradation Product (DP1) at 6.359 RT with 13.32% degradation. QbD study shows there is no effect of degradation condition on retention time with little effect on the peak area of alectinib.

Alkali Hydrolysis

The alkali degradation for the above method software suggested conditions were experimentally conducted, and results were found out with no degradation.

Peroxide degradation

The peroxide degradation for above method software suggested conditions were experimentally conducted and results found out with one (DP2) at 7.364 min RT and 49.57% degradation. QbD study shows there is no effect of degradation condition on retention time, with little effect on peak area of alectinib.

Table 5: Summary of stress degradation.

Sl. No.	Stress condition	Treatment	Degradation products with RT in min	% Degradation
1.	Acidic degradation	1N HCl at 40°C and 60°C reflux for 30 min, for 1 to 5 days.	DP1 at 6.359	13.32%
2.	Alkaline degradation	1N NaOH at 40°C and 60°C reflux for 30 min, for 1 to 5 days.	-	-
3.	Oxidative degradation	20% H_2O_2 at 25° to 60°C for 30 min. for 1 to 5 days	DP2 at 7.364min	49.57%
4.	Thermal degradation	kept in oven 30°C to 105°C for 6 hr from 1 to 5 days	DP3 at 9.647	39.68%
5.	Photolytic degradation	UV light of 200-Watt h/m^2 Light 1×ICH and 3×ICH for 1 to 5 days	DP4 at 12.441 min	18.95%

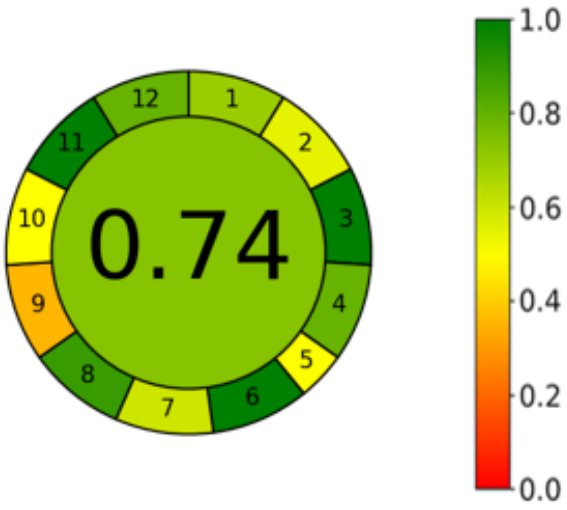

Figure 4: Method greenness assessment.

Thermal degradation

Thermal degradation study has found out with degradation product DP3 at 9.647 min RT, and percentage degradation was found to be 39.68%. QbD study shows no effect of storage condition and temperature on RT of alectinib.

Photolytic degradation

Photolytic degradation of alectinib was found out with DP4, which was obtained at 12.41 retention time with a percentage degradation of 18.95%. In this case also there is no effect of storage condition and temperature on retention of alectinib with little effect on peak area of alectinib.

Statistical Analysis

The statistical value for optimal design optimization of the aforementioned research indicates ANOVA values are significant since the *p*-value is less than 0.005, as shown in Table 2. Shows model significance (Kumari *et al.*, 2024).

Comparison with previous literature

The previously disclosed approach by Suchithra for estimating alectinib has a longer retention time of 5.8 min. Rutuja *et al.*, discovered a method for estimating alectinib with a longer retention duration, 8.6 min. The current study quantifies alectinib at 3.212 min. Maithani *et al.*, validated alectinib using buffer and acetonitrile as mobile phases at a 55:45 v/v concentration. While the current approach uses water and methanol as a mobile phase, it is more cost effective than acetonitrile and buffer. Mohamed *et al.*'s alectinib stability study does not include a QbD analysis for the stability-indicating method. As a result, the current study of alectinib with QbD implementation for optimization and stability analysis revealed more profound findings with detailed force degradation analysis.

Greenness of method

Greenness Score using AGREE (0.74) Figure 4, for the current method with evaluation of environmental sustainability finds with a greater score near to 1.0. Signifies an eco-friendly method for alectinib estimation.

CONCLUSION

A new, simple, precise, and robust green metric-based RP-HPLC method for alectinib estimation was optimized and validated using QbD analysis with predefined parameters. Tests showed that alectinib breaks down easily in acidic, oxidative, heat, and light conditions, but it was found stable in alkaline conditions. This method can be used for quality control analysis of alectinib.

ACKNOWLEDGEMENT

The authors are grateful to Smt. Kashibai Navale College of Pharmacy, Kondhwa for providing the facility to carry out this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography, **QbD:** Quality by Design, **LOD:** Limit of Detection, **LOQ:** Limit of Quantification, **RT:** Retention Time, **RSD:** Relative Standard Deviation, **ICH:** International Council for Harmonization, **DP:** Degradation Product.

REFERENCES

Bairagi, A., Kothrukar, R., Chikhale, H. *et al.* (2024). AQbD-Novel strategy for analytical methods. Future Journal of Pharmaceutical Sciences, 10, 1. https://doi.org/10.1186/s43094-024-00689-6

Beardslee, T., & Lawson, J. (2018). Alectinib and brigatinib: New second-generation ALK inhibitors for the treatment of non-small cell lung cancer. Journal of the Advanced Practitioner in Oncology, 9(1), 94–101. https://doi.org/10.6004/jadpro.2018.9.1.9

Chowdary, G. L., Ravisankar, P., Kumar, G. A. *et al.* (2020). Analytical method validation parameters: An updated review. International Journal of Pharmaceutical Sciences Review and Research, 61(2), 1–7.

Durga, K., Mrunal, D., Prajakta, P., & Jagadish, P. (2022). Evaluation of pH-dependent solubility and examination of variation in pharmacokinetic properties of alectinib: A quantitative study by implementing integrated quality by design approach for RP-HPLC method development and optimization. Indian Journal of Pharmaceutical Education and Research, 56(4), 56–65. https://doi.org/10.5530/ijper.56.4.9

Frederick, V., & Alireza, K. (2011). Development of quality-by-design analytical methods. Journal of Pharmaceutical Sciences, 100(3), 797–812. https://doi.org/10.1002/jps.22336

Gamal, M., Naguib, I. A., Panda, D. S., & Abdallah, F. F. (2021). Comparative study of four greenness assessment tools for selection of greenest analytical method for assay of hyoscine N-butyl bromide. Analytical Methods: Advancing Methods and Applications, 13(3), 369–380. https://doi.org/10.1039/d0ay02169e

Gunturu, R., & Kantipudi, R. (2022). A study of stability indicating development and validation of a method for simultaneous estimation of brigatinib and alectinib using reverse-phase ultra-performance liquid chromatography in active pharmaceutical ingredient form. Journal of Pharmaceutical Research International, 34(9A), 46–55. https://doi.org/10.9734/jpri/2022/v34i9A35418

Harish, V., Almalki, W. H., Alshehri, A., Alzahrani, A., Gupta, M. M., Alzarea, S. I., Kazmi, I., Gulati, M., Tewari, D., Gupta, G., Dua, K., & Singh, S. K. (2023). Quality by design (QbD) based method for estimation of xanthohumol in bulk and solid lipid nanoparticles and validation. Molecules, 28(2), Article 472. https://doi.org/10.3390/molecules28020472

International Council for Harmonization. (2009). ICH harmonized tripartite guideline: Pharmaceutical development Q8, R2. International Council for Harmonization.

International Council for Harmonization. (2022). ICH harmonized guideline: Analytical procedure development, Q14. International Council for Harmonization.

Jadhav, J. B., Girawale, N. N., & Chaudhari, R. A. (2014). Quality by design (QbD) approach used in development of pharmaceuticals. International Journal of Pure and Applied Bioscience, 2(5), 214–217.

Koradia, S., Patel, M., Sen, A. K., Sen, D. B., & Pradhan, P. (2024). Analytical quality by design-based thin-layer chromatography method development and validation for assay and content uniformity testing of the anti-neoplastic drug axitinib in tablet formulation. Separation Science Plus, 7(3), Article 23001. https://doi.org/10.1002/sscp.202300176

Kumari, D. S., Sunitha, P., Kuchana, V., & Kalyani, G. (2024). Stability indicating RP-HPLC method development and validation for the estimation of alectinib in bulk form and pharmaceutical dosage form. World Journal of Pharmaceutical and Pharmaceutical Sciences, 13(11).

Lee, S., Nath, C. E., Balzer, B. W. R., Lewis, C. R., Trahair, T. N., Anazodo, A. C., & Shaw, P. J. (2020). An HPLC–PDA method for determination of alectinib concentrations in the plasma of an adolescent. Acta Chromatographica, 32(3), 166–169. https://doi.org/10.1556/1326.2019.00578

Manohar, S. K., Gowrav, M. P., & Gowd, D. V. (2022). QbD-based development of orodispersible films of antipsychotic drugs. International Journal of Applied Pharmaceutics, 14(5), 41–52.

Meshram, P., & Ranpise, N. (2024). Advanced green chemistry, HPLC method development, and validation: Integrating stability indicating, quality by design (QbD), in ferulic acid and its application in NLCs-based nanoformulation. Analytical Chemistry Letters, 14(6), 909–934. https://doi.org/10.1080/22297928.2024.2428933

National Cancer Institute. (2017). Alectinib approved by FDA for untreated lung cancer. Retrieved April 18, 2025, https://www.cancer.gov/news-events/cancer-currents-blog/2017/alectinib-fda-untreated-lung-cancer

National Center for Biotechnology Information. (2024). PubChem compound summary for CID 49806720, Alectinib. Retrieved April 18, 2025, https://pubchem.ncbi.nlm.nih.gov/compound/Alectinib

Nhavale, G., Gudge, R., Patel, A., & Barde, K. (2024). Quality by design approach for the stability indicating method development and validation of selpercatinib drug formulation by using RP-HPLC. International Journal of Pharmaceutical Quality Assurance, 15(2), 580–587. https://doi.org/10.25258/ijpqa.15.2.04

Patel, K. Y., Dedania, Z. R., Dedania, R. R., & Patel, U. (2021). QbD approach to HPLC method development and validation of ceftriaxone sodium. Future Journal of Pharmaceutical Sciences, 7(1), 141. https://doi.org/10.1186/s43094-021-00286-4

Pavani, B., Belide, P., & Mounica, P. (2019). RP-HPLC method development and validation for estimation of alectinib in bulk and pharmaceutical dosage form. International Journal of Pharmaceutical Analysis Research, 8(3), 293–300. https://doi.org/10.61096/ijpar.v8.iss3.2019.293-300

Peraman, R., Bhadraya, K., & Padmanabha Reddy, Y. (2015). Analytical quality by design: A tool for regulatory flexibility and robust analytics. International Journal of Analytical Chemistry, 2015, Article 868727. https://doi.org/10.1155/2015/868727

Prashanthi, Y., Rao, T. N., & Srinivas, Y. (2018). Method development and validation of alectinib drug by RP-HPLC in bulk and pharmaceutical dosage form. Asian Journal of Pharmaceutical Analysis, 8(4), 186–190. https://doi.org/10.5958/2231-5675.2018.00034.0

Samacá-Samacá, D., Prieto-Pinto, L., Pérez, A. Y., Valderrama, C., & Hernández, F. (2023). Alectinib for treating patients with metastatic ALK-positive NSCLC: Systematic review and network meta-analysis. Lung Cancer Management, 12(2), Article LMT59. https://doi.org/10.2217/lmt-2022-0018

Sharma, S., Naman, S., Goyal, K., & Bald, A. (2023). Simultaneous estimation of atovaquone and mefloquine hydrochloride: QbD based method development and validation. Indian Journal of Pharmaceutical Education and Research, 57(1), 1–15. https://doi.org/10.5530/ijper.57.1.1

Validation of analytical procedures: Text and methodology Q2. (2005) International Conference on Harmonisation, R1. ICH. Harmonized Tripartite Guideline.

van Veelen, A., van Geel, R., Schoufs, R., de Beer, Y., Stolk, L. M., Hendriks, L. E. L., & Croes, S. (2021). Development and validation of an HPLC–MS/MS method to simultaneously quantify alectinib, crizotinib, erlotinib, gefitinib, and osimertinib in human plasma samples using one assay run. Biomedical Chromatography, 35(12), Article e5224. https://doi.org/10.1002/bmc.5224

Vora, R., & Shah, Y. (2019). Investigation of quality target process parameters (QTPP) and critical material attributes (CMA) of nanocellulose as a potential excipient. International Journal of Applied Pharmaceutics, 11(4), 386–395. https://doi.org/10.22159/ijap.2019v11i4.33656

Cite this article: Tangde J, Sawant S. New RP-HPLC Method Development and Validation, Incorporating Quality by Design (QbD) with Force Degradation Study for Alectinib Estimation: A Green Approach. Int. J. Pharm. Investigation. 2026;16(1):201-9.