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Method Development and Validation for The Quantitative Estimation of Alectinib in Bulk Form and Marketed Pharmaceutical Dosage Forms by Using RP-HPLC

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Alectinib in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Phenomenex Luna ODS C_{18} (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (25:75%) v/v as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Alectinib was found to be 2.265min. The calibration plot was linear over the concentration range of 6-14 µg mL^{-1} with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Alectinib in bulk and marketed pharmaceutical dosage form dosage form.

KEY WORDS: Alectinib, RP-HPLC, Validation, Accuracy, Precision, ICH Guidelines.

INTRODUCTION

Alectinib is a second-generation oral drug that selectively inhibits the activity of anaplastic lymphoma kinase (ALK) tyrosine kinase. It is specifically used in the treatment of non-small cell lung cancer (NSCLC) expressing the ALK-EML4 (echinoderm microtubule-associated protein-like 4) fusion protein that causes proliferation of NSCLC cells[1]. Inhibition of ALK prevents phosphorylation and subsequent downstream activation of STAT3 and AKT resulting in reduced tumour cell viability. Approved under accelerated approval in 2015, Alectinib is indicated for use in patients who have progressed on or were not tolerant of Crizotinib, which is associated with the development of resistance. Alectinib is a kinase inhibitor indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive, metastatic non-small cell lung cancer (NSCLC) who have progressed on or are intolerant to Crizotinib[2]. This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials. Alectinib is used to treat a certain type of non-small-cell lung cancer (NSCLC) that has spread to other parts of the body. Alectinib is in a class of medications called kinase inhibitors[3]. It works by blocking the action of an abnormal protein that signals cancer cells to multiply. The IUPAC name of Alectinib is 9-ethyl-6, 6-dimethyl-8-(4-morpholin-4-yl piperidin-1-yl)-11-oxo-5H-benzo [b] carbazole-3-carbonitrile.

The Chemical Structure of Alectinib is shown in following Figure-1.

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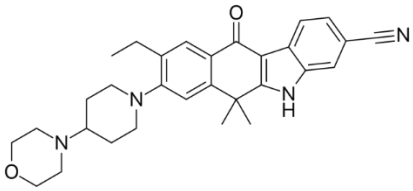

Fig-1: Chemical Structure of Alectinib

EXPERIMENTAL

Instruments Used:

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Chemicals Used:

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Alectinib	Synpharma Research Lab, Hyderabad
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Alectinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[27].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

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Optimization of Column:

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow[4].

Preparation of Buffer and Mobile Phase:

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of Mobile Phase:

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicate for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration[5].

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Alectinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC[6]. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.1ml of the above Alectinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Alectinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

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Further pipette 0.1ml of Alectinib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula[7]:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (6ppm of Alectinib):

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (8ppm of Alectinib):

Take 0.8ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (10ppm of Alectinib):

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (12ppm of Alectinib):

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (14ppm of Alectinib):

Take 0.14ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[8].

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Precision

Repeatability

Preparation of Alectinib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Alectinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[9].

Accuracy:

For Preparation of 50% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Alectinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

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Further pipette 0.1ml of the above Alectinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 150% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Alectinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Alectinib and calculate the individual recovery and mean recovery values[10].

Robustness:

The analysis was performed in different conditions to find the variability of test results.

The following conditions are checked for variation of results.

For Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Alectinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Alectinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 30:70, 20:80 instead (25:75), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded[11].

RESULTS AND DISCUSSION

Analytical Method Development:

Mobile phase ratio	: Methanol: Phosphate Buffer (25:75%) v/v
Column	: Phenomenex Luna ODS C_{18} (4.6×250mm, 5µm)
Column temperature	: Ambient

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Wavelength	: 235nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 8min

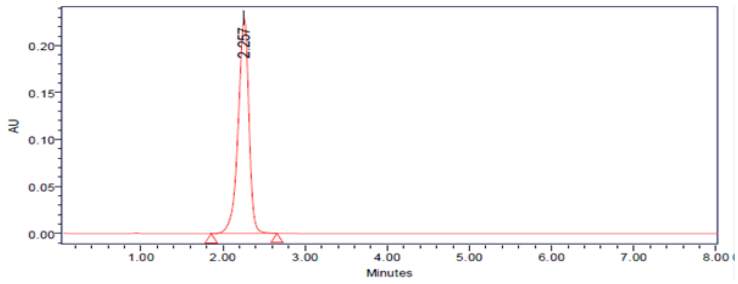

Fig-2: Optimized Chromatographic Condition

Validation of Analytical Method:

The developed chromatographic method was validated for Specificity, Linearity, Precision, Accuracy, Sensitivity, Robustness and System suitability[12-15].

System Suitability

Prepared standard solution (5 injections) and evaluated system suitability parameters as per test method[16].

Table-3: Results of System Suitability for Alectinib

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Alectinib	2.277	1652847	185647	6589	1.24
2	Alectinib	2.277	1653658	186254	6587	1.26
3	Alectinib	2.267	1654521	185475	6584	1.28
4	Alectinib	2.265	1653564	186594	6582	1.29
5	Alectinib	2.277	1658745		6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
% RSD			0.142371			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components[17]. Analytical method was tested for specificity to measure accurately quantitates Alectinib in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100 = 99.40\%$$

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The % purity of Alectinib in pharmaceutical dosage form was found to be 99.40%.

Linearity

Linearity test solutions for Alectinib and its impurities are prepared by diluting stock solutions to the required concentrations. The solutions are prepared at different concentration levels and injected into the column under the chromatographic conditions developed[18]. The data of peak area versus concentration was subjected to least square regression analysis.

Chromatographic Data for Linearity Study:

Table-4: Data for Linearity of Alectinib

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

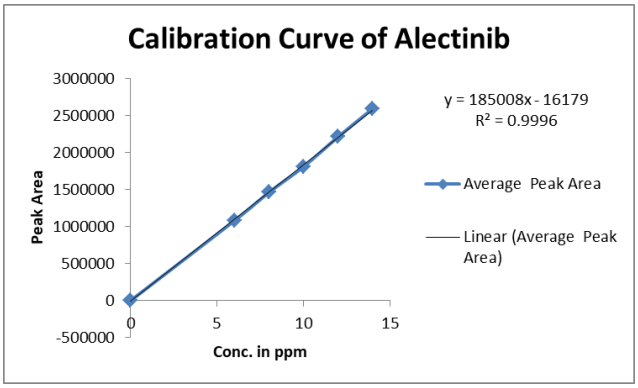

Fig-3: Linearity Curve of Alectinib

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Alectinib is a straight line.

Y = mx + c

Slope (m) = 18500

Intercept (c) = 16179

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 0.16179. These values meet the validation criteria.

Precision:

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The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions[19].

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-5: Results of Repeatability for Alectinib:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Alectinib	2.293	1658954	186958	1.26	6785
2	Alectinib	2.276	1658745	187548	1.27	6854
3	Alectinib	2.286	1659865	189854	1.26	6852
4	Alectinib	2.277	1653254	186985	1.25	6784
5	Alectinib	2.280	1654781	189542	1.24	6895
6	Alectinib	2.293	1661324	187586	1.28	6965
Mean			**1657821**			
Std. Dev			**3120.433**			
%RSD			**0.188225**			

Intermediate Precision:

Analyst1:

Table-6: Results of Intermediate Precision for Alectinib

S. No.	Peak Name	RT	Area	Height	USP Plate	USP Tailing
1	Alectinib	2.274	1678541	186589	6587	1.26
2	Alectinib	2.258	1685985	186598	6321	1.26
3	Alectinib	2.267	1685745	186985	6385	1.25
4	Alectinib	2.270	1685987	187854	6580	1.26
5	Alectinib	2.264	1698526	187549	6721	1.27
6	Alectinib	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std. Dev.			6463.466			
% RSD			0.383182			

Analyst 2:

Table-7: Results of Intermediate Precision Analyst 2 for Alectinib

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Alectinib	2.277	1665847	167481	6854	1.25
2	Alectinib	2.255	1658989	167854	6785	1.26
3	Alectinib	2.265	1659845	167895	6854	1.24
4	Alectinib	2.255	1665964	167854	6895	1.26
5	Alectinib	2.253	1659863	168585	6459	1.25
6	Alectinib	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std. Dev.			3501.766			

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% RSD			0.210601			

Accuracy: The degree of closeness between a test value and the realistic value is called accuracy. By collecting at least nine measurements across three concentration levels, accuracy was assessed. The acceptance criterion for accuracy is the RSD for all recovery values should not be more than 2%. Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated[20].

Table-8: The Accuracy Results for Alectinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[21].

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: = 0.95µg/ml

Quantitation Limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined[22].

LOQ=10×σ/S

Where

σ = Standard deviation of the response
S = Slope of the calibration curve

Result: = 2.9µg/ml

Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Alectinib. The method is robust only in less flow condition. The standard of Alectinib was injected by changing the conditions of chromatography[23-26]. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-9: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

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SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 235 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Phenomenex Luna ODS C_{18} (4.6×250 mm, 5µm) because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer pH-3.6 in the ratio of 25:75% v/v was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 8min because analyze gave peak around 2.265min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of the Alectinib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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