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A Novel Ultra Performance Liquid Chromatography-PDA Method Development and Validation for Alectinib in Bulk and its Application to Tablet Dosage Form

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ABSTRACT

Objectives: To develop a novel ultra-performance liquid chromatographic technique for the estimation of alectinib in a API and tablet. **Methods:** The chromatographic separation was achieved using DIKMA Endoversil (2.1 x 50mm, 1.7µm) column, mobile phase was phosphate buffer, pH 4.6 and methanol as a mobile phase (45:55) with a flow rate of 0.4 mL/min and eluent was monitored at 265 nm. The method was continued and validated in accordance with International conference on harmonization guidelines. Validation study revealed the specificity and reliability of the method. **Results:** In this method alectinib was eluted with retention time of 0.418 min. Calibration curve plots were found linear over the concentration ranges 1-100 µg/mL for alectinib. Limit of detection was 0.015µg/ml and limit of quantification was found 0.07µg/mL. The % assay of the marketed dosage form was found 97.80 %, even the present approach was found to be effective in the analysis of alectinib in force degradation condition. **Conclusion:** The experiential evidences of all the study results revealed the suitability of the estimation of alectinib in API and tablet formulation.

Key words: Alectinib, UPLC, Method Development, Method Validation, ICH Guidelines.

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INTRODUCTION

Lung cancer is a type of cancer that starts in the lungs. Cancer starts when cells in the body start to raise out of control. About 82% to 86% of lung carcinoma are NSCLC. The chief subtypes of NSCLC are squamous cell carcinoma, adenocarcinoma and large cell carcinoma. These subtypes are grouped together as NSCLC, starting from various types of lung cells, since their care and prognosis (outlook) are often similar.[1] Alectinib is an organic heterotetracyclic compound that is 6,6-dimethyl-5,6-dihydro-11H-benzo[b]carbazol-11-one carrying additional cyano, 4-(morpholin-4-yl)piperidin-1-yl and ethyl substituent at positions 3, 8 and 9 respectively (Figure 1).[2] Alectinib is an orally accessible anaplastic lymphoma kinase (ALK) inhibitor of the tyrosine kinase receptors with anticancer properties. Alectinib binds to and inhibits ALK kinase upon administration. The inhibition leads to disruption of ALK-mediated signalling and eventually inhibits the growth of tumer cell in ALK-overexpressing tumor cells.[3] The FDA approved alectinib (Alecensa) for frontline therapy in patients with ALK-positive metastatic NSCLC, a TKI targeting ALK and RET, in November 2017. In addition to offering this new indication, the FDA has also converted the accelerated approval of alectinib to full approval for patients with ALK-positive NSCLC who have progressed to crizotinib.[4] Extensive literature review for the alacetinib estimation method using different analytical tools was conducted, it was disclosed that only two HPLC methods,[5,6] are available for the alectinib estimationin tablet dosage form. Two LCMS methods[7,8] for the estimation of alectinib in human plasma and their pharmacokinetic study were also published. There were no UV spectrophotometries and other methods available for this study. In one HPLC method developed by Pavani *et al.* reported the retention time of alectinib is 2.302 min and used water: acetonitrile in the ration of 50:50 as a mobile phase. But in the alectinib assay they used doxorubicin 150 mg of marketed dosage form, so this procedure for alectinib is entirely unjustified. In another report produced by Prashyanthi *et al.* 1.9 min retention time was recorded and methanol and phosphate buffer (pH 3.0) is used a mobile phase. For the assay they used 10 mg of alectinib tablet as a marketed dosage form, but alectinib 10 mg dosage form is not available in any market around the world and even 10 mg as a single dosage form is not feasible, because the minimum dose of alectinib is 150 mg. Hence the question arises about the credibility of the method established and there is a huge uncertainty about the validity of both the methods mentioned. Taking into consideration the above information, we considered the significance and necessity of a reliable, accurate and precise validated method for estimating alectinib in both bulk and tablet dosage.

The present research work has therefore been designed to reduce the shortcomings of the previously mentioned methods and to establish a reliable and accurate stability indicating the UPLC process.

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade working standards Alectinib (99.97%) was procured from Hetero Pharma Ltd, Hyderabad, India. The tablets of alectinib were collected from the Chittaranjan National cancer Institute, Kolkata, India. All required chemicals and reagents were purchased from Finer chemical Ltd, Fisher Scientific and Merck.

Instrumentation Conditions

The analysis was performed using Ultra performance liquid chromatography (UPLC) Acquity Waters, PDA detector. Software:

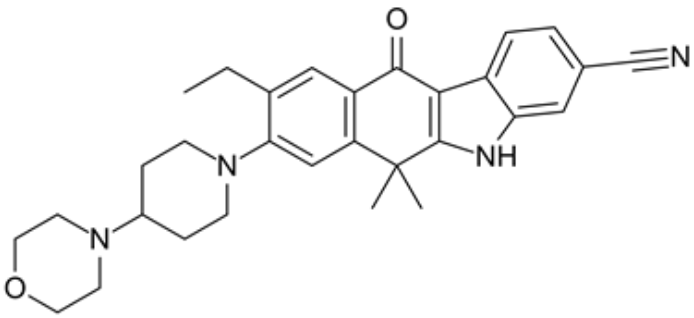

Figure 1: Chemical structure of alectinib.

Empower 2 equipped with auto sampler and PDA detector. The analytical balance 0.1mg sensitivity (Afcoset ER-200A), pH meter (Adwa – AD 1020), Ultra Sonicator were used. The column used was DIKMA Endoversil (2.1 x 50mm, 1.7µm) UPLC column with the flow rate 0.4 ml/min (isocratic) Detection was carried out at 265 nm.

Preparation of 0.05 M phosphate buffer

About 6.8043 g of potassium dihydrogen orthophosphate was weigh and transfer in to a 1000 ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 4.6 with orthophosphoric acid.

Preparation of mobile phase

Accurately measured 450 ml (45%) of above buffer and 550 ml of HPLC grade methanol (55%) were mixed well and degassed in an ultrasonic water bath for 10 min and then filtered through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation

Accurately weigh and transfer 25mg of alectinib working standard into a 25ml clean dry volumetric flask add about 10ml of methanol and sonicated to dissolve it completely and make volume up to the mark with the mobile phase to attain 1 mg/mL concentration. The working solution of alectinib was prepared by further pipette 2 ml of stock solutions into a volumetric flask of quantity 100 ml and further the solution was dilute to the mark using the diluent to attain 20 µg /mL of alectinib.

Assay of marketed dosage form

Accurately weigh 10 tablets and triturated in a mortar and pestle and transfer equivalent to 100 mg alectinib (153.33 mg of tablet Powder) sample into a 100 ml clean dry volumetric flask add about 10 ml of warm methanol and sonicated it up to 30 mins to dissolve completely and make volume up to the mark with the mobile phase. Then it is filtered through 0.44 micron injection filter which is considered as stock solution. Using a micropipette to pipette 0.2 ml of solution from the above stock solution into a 10 ml volumetric flask and dilute it with diluent up to the level. 10 µL of both sample and standard solution was kept in auto sampler for injection, in triplicate into the chromatographic system. The areas for alectinib peak were measured and assay percentage was calculated.

Method Validation

Specificity

It was performed using placebo interference test of the sample solution using 500 mg of placebo, which is analogous to one tablet dissolved in 100ml of mobile phase and the placebo solution was treat like a standard solution. The solution was injected to the chromatographic system to assess the possible interfering peaks.

System suitability

This study was conceded[9] to rationalize whether analytical system is running properly. It was conducted by injecting the six replicates of standard solution of alectinib. The %RSD of the a range of optimised parameters like peak area, theoretical plates, retention time and asymmetric factor were calculated.

Accuracy

To validate the accuracy of the present method recovery study was conducted at different levels (80%, 100% and 120%) of pure alcetinib. The amounts of standard alcetinib was added to a fixed concentration to alcetinib tablet sample solution to attain the various levels. This study[10] were carried out three times and the percentage recovery as well as percentage mean recovery was calculated.

Intraday and Inter day precision

The precision of the method was[11] evaluated by analysing the six sample solutions in triplicate (n=6) of 10µg/mL of alectinib solution. The intra- and inter-day precision was determined by analysing for six times on the same day (intra-day study) and repeated on the second and also third day (inter-day study). The chromatograms were recorded, peak area and retention time of alectinib was determined and relative standard deviation (RSD) was calculated.

Detection and Quantitation limit

The limit of detection is characterize as the concentration for which a signal-to-noise ratio of 3 was obtained and for Quantitation limit, a signal-to-noise ratio of 10 was considered.[12] Standard solution of alectinib was prepared by chronological dilution and injected in to the chromatographic system in declining order of concentration in the range of 0.01-10µg/ml of alectinib.

Linearity

To perform the linearity study, standard solution of alectinib were prepared as describe earlier, different volumes of aliquot from the standard solution were diluted with mobile phase to achieve different concentrations in the range of 1-100 µg/ml of alectinib. Calibration curve was plotted for alectinib considering concentration versus peak area, obtained data was subjected to regression analysis.

Robustness

Analysis of the robustness of the system established[12] by deliberately changing the chromatographic condition. Six sample solutions were prepared and analysed in triplicate utilising the optimised condition by varying the analytical conditions like flow rate, mobile phase ratio and detection wavelength at three different levels. All the optimised parameter was found within the limit. For the calculation of percentage RSD the tailing factor was considered.

Force degradation study of darunavir

Force degradation study of the alectinib solution[13] was conducted using ICH prescribed stress condition such as acidic, alkaline, oxidative, thermal and photolytic stress conditions. All the types of degradation studies have been performed in triplicate and mean peak area has been considered for the calculation.

Acid degradation

The acid degradation study was performed using environmental test chamber (Acamus Technologies, India) at 60°C and 75% relative humidity using 1m HCl. 0.5 ml of stock solution(1 mg/mL) was taken in 10 ml of volumetric flask, 0.5 ml of 1m HCl was added to the flask, kept in environmental test chamber for 16 hr. After the suitable stress period the solution was neutralized using 1m NaOH and make up the volume with mobile phase and injected in to the UPLC system

Alkaline degradation

This study was performed at 60°C and relative humidity (58%) using similar environmental chamber. Accurately 0.5 ml of stock solution was taken in 10 ml volumetric flask mixed with 1m 0.5 ml of 1m NaOH and kept for 16 hr. After the suitable stress period. The solution was neutralised with 1m HCl and the mobile phase volume was formed and injected into the UPLC device.

Oxidative degradation

It was performed in the same environmental chamber at 40°C, 75% relative humidity. The 6% H_2O_2 was used to execute the oxidation the studied sample. For this purpose 0.5 ml of stock solution was taken volumetric flask (10 mL) and 0.5 ml of 6% H_2O_2 was added in to flask and kept at 60°C for 16 hr, finally make up the volume up to mark with mobile phase and injected in to the UPLC system.

Thermal degradation

It has been carried out in the environmental chamber at 40°C, 75% relative humidity and using the oven at 105°C. Accurately 0.5 ml of stock solution was placed in a 10 ml volumetric flask and held in the chamber for 144 hr and 1 mg of dry solid pharmaceutical was put in the oven at 110°C for 2 days for dry heat thermolysis.

Photolytic degradation

This study was carried out in the sunlight (60000- 70000 lux) during day time for the period of 48 hr. Accurately 0.5 ml of the stock solution was transferred in to the flask and volume was made up to the level using mobile phase solution and used for the study.

RESULTS

Method Development

Several chromatographic conditions were investigated to attain a novel UPLC method for alectinib estimation in API and tablet formulation. Numerous parameters such as mobile phase composition, column type, pH of mobile phase and diluents were considered during trials. Various proportions of solvents, buffer, were tested in order to attain suitable composition of the mobile phase for the method optimization. Finally alectinib was eluted with good peak shape and very low retention time with the mobile phase phosphate buffer, pH 4.6 and methanol as a mobile phase (45:55) with a 0.4 mL / min flow rate. The retention time 0.418 min was observed for the alectinib with PDA detection at 265 nm. As per ICH guidelines, the established technique was validated. Figure 2 showed an integrated chromatogram.

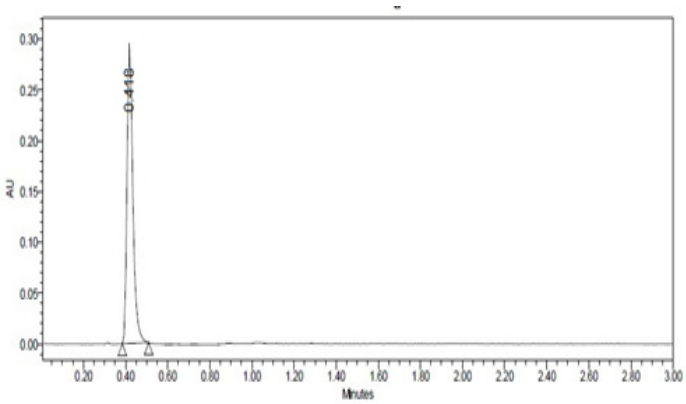

Figure 2: Optimized UPLC Chromatogram of alectinib.

Method validation

The optimized method for alectinib bulk was effectively applied for the subsequent validation studies. The result of the assay of marketed tablet dosage form of alectinib shows the average percentage purity was 97.80 %. The assay chromatogram was depicted in Figure 3 and result was revealed in Table 1.

In the specificity study no excipients peaks were found at the analyte the retention time. System Suitability study was conducted to confirm the proper operational of the equipment used for analytical measurements. Several parameters like tailing factor and theoretical plates were taken into consideration. The % relative standard deviation of peak area, theoretical plates, tailing factors and retention time were 0.32 %, 0.98 %, 1.57% and 0.73 % respectively.

Both accuracy and precision were determined with standard quality control samples. The results of accuracy as a mean % recovery was found 99.38 and the % RSD was not more than 2 %, shown in Table 2. The % RSD of the intra and inter day precision study was found 0.83 and 1.32. The results of precision study were also shown in Table 2. The linearity was determined for the range of concentrations 1-100 µg/ml. and the correlation coefficient was obtained 0.997 for the alectinib mentioned in

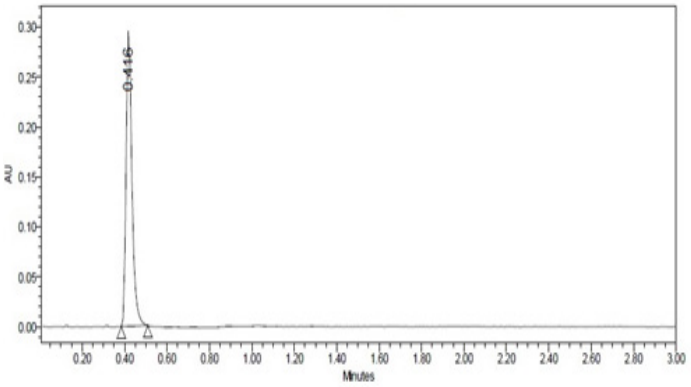

Figure 3: UPLC Chromatogram of the assay of marketed dosage from.

Table 1: Assay of marketed formulations.

Formulation	Labeled claimed	Amount obtained*	Percentage purity*
Alecnib tablets (150 mg alectinib), manufactured by Everest Pharmaceutical ltd, Nepal.	150mg	146.71 mg	97.80%

*average of three replicates

Table 2: Summary of validation parameters.

Parameters	Alectinib
Linearity range (mg/ml)	1-100
Co-relation co-efficient	0.998
LOD µg/ml	0.015
LOQ µg/ml	0.070
Intraday precision (% RSD)	0.83
Inter-day precision (% RSD)	1.32
Mean % recovery in accuracy	99.38
% RSD of tailing factor for robustness study	1.09

Table 2. The overlay chromatogram of the linearity was shown in Figure 4. The detection and quantitation limit were found to 0.015 µg/ml and 0.07 µg/ml respectively. Robustness study of the method was carried out by changing three parameters from the chromatographic conditions such as changes in mobile phase composition (±3%), changes in flow rate (±0.1ml/min) and detection wavelength (±2 nm) and the % RSD of the tailing factor which was considered as a tool parameter was establish to be fewer than 1.09 as shown in Table 2. Degradation studies of alectinib were performed under the influence of acid, alkali, oxidation, thermal, photolytic conditions. Degradation was found almost all stressed condition except photolytic influence. Acidic stressed condition shows 5.18%, alkaline stress condition shows 3.10 %, peroxide condition shows 3.29%. The thermal degradation shows 4.13 % degradation respectively, the results were revealed in Table 3 and chromatograms shown in Figure 5.

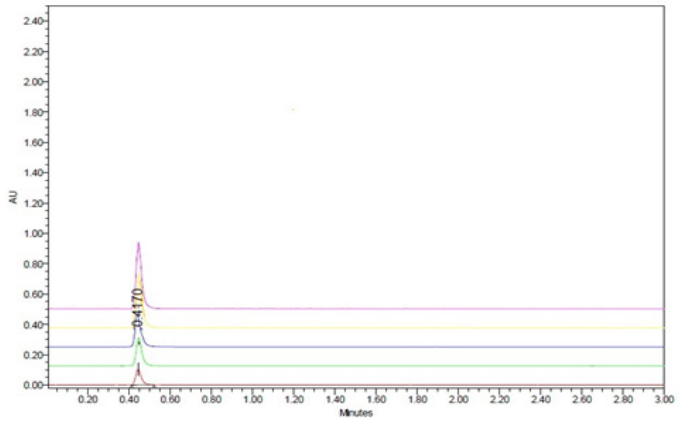

Figure 4: Overlay UPLC chromatogram of linearity study of alectinib.

Table 3: Degradation results for alectinib solution.

Sample Name	Alectinib				
	Mean Area*	% Degraded	Purity Angle	Purity Threshold	Peak purity
Acid	463253	5.18	1.36	2.19	Passes
Base	470923	3.10	0.72	1.32	Passes
Peroxide	461775	3.29	0.84	2.43	Passes
Photo	481303	0.63	0.08	1.02	Passes
Thermal	471261	4.13	1.48	3.13	Passes

DISCUSSION

In the present research work the optimized chromatographic conditions was established after several trials. By utilizing the developed optimized condition alectinib was eluted with a good peak shape, very short retention time using a mixture of methanol and phosphate buffer in the volume ratio of 55:45 with a flow rate of 0.4 mL/min. The retention time obtained for alectinib is 0.418 min. Accuracy and precision were determined as per the guidelines and the % recovery was found as a result of accuracy within the acceptable limit i.e. within 95-105% as shown in the result, confirms the accuracy of the developed method. In the intraday and interday precision study, the amount found was calculated and calculated %RSD was found satisfactory and within the limit. The results of precision study indicated that the method developed was found to be specific. The percentage assay in marketed dosage form 97.80 %, which indicates the suitability of the developed method to analyze alectinib in marketed dosage form. In the linearity study of the method the correlation coefficient was found near to 0.997 for alectinib which indicates its specified linearity. The least squares method

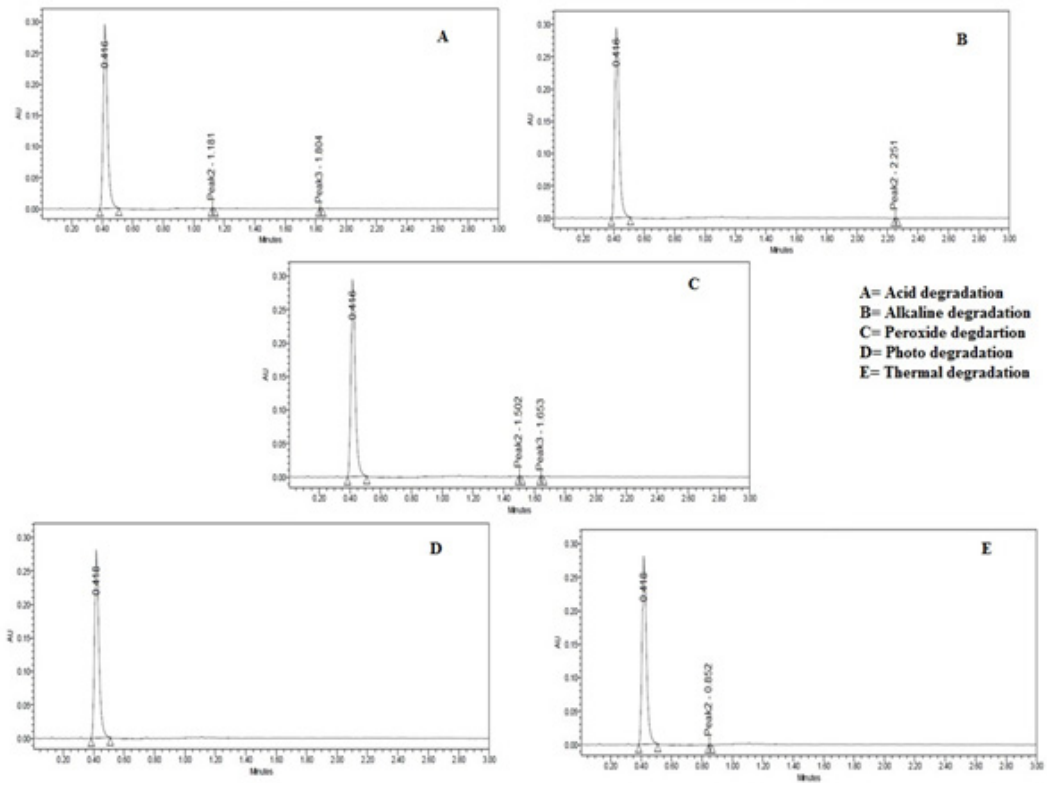

Figure 5: Degradation chromatograms of alectinib using different stressed condition.

was used to establish the regression line and the curve was found linear. The limit of detection and quantitation values proved sensitivity of the developed method and in 0.07 µg/ml concentrations also alectinib can be quantifiable. In the specificity result it was found that no excipients peaks were found at the retention time of the alectinib and justifies the specificity of the method. In the robustness study, the tailing factor was considered for the measurement tool and the % RSD of the tailing factor was found less than 2.0 validating the robustness of the established approach, because no significant changes were obtained on deliberate changes in the optimized parameters. The result of degradation studies of alectinib indicated that acidic and thermal stressed condition leads to little more degradation in compare to other stressed condition, whereas in photolytic condition no degradation was establish. In the every stressed conditions, the chromatograms of alectinib was found very specific.

CONCLUSION

Based on the experiential evidences of the present developed method for the alectinib, authors are strongly declare about the novelty of the method over the available very few methods. The present UPLC method which is 'rapid' because it significantly reduced the total analysis time within 0.418 min, which can consider lowest analysis time required. The present method is "stability indicating" because not as much of degradation was observed in stressed conditions and good separation of alectinib among the other degraded peaks also observed. Under the acceptance criteria of ICH Q2B guidelines, the results of the validation parameters were noted. Hence the present developed method can be employed as a novel, trustworthy, validated method can be apply for routine analytical and quality control assay of alectinib in the bulk as well as tablet dosage form.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ABBREVIATIONS

UPLC: Ultra performance liquid chromatography; **ICH:** International conference on harmonization; **PDA:** Photo diode array; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **SD:** Standard deviation; **RSD:** Relative standard deviation; **API:** Active Pharmaceutical Ingredient.

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