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Analytical Method Development and Validation of Alectinib by RP-HPLC Technique

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ABSTRACT

A simple and precise RP-HPLC method for the estimation of Alectinib in tablet dosage form was developed and validated. The chromatographic separation of the drug was achieved with a C18 column (250 mm × 4.6 mm i.d., particle size 5µ) using buffer and acetonitrile (55:45 v/v) as the mobile phase. The buffer used in the mobile phase contained 50mM ammonium acetate and its pH was adjusted to 5.8±0.02. The instrument was set at flow rate of 1.1 $mLmin^{-1}$ at ambient temperature and the wavelength of the UV-visible detector at 292nm. The method showed excellent linearity over a range of 10-70 μgmL^{-1} for the drug. The correlation coefficient for Alectinib was noted to be 0.9994. The mean recovery values were found to be 99.62% and 100.18%. The proposed method could be suitable for the quantitative determination of Alectinib in pharmaceutical dosage forms and also for quality control in bulk manufacturing. The *f*-test and *t*-test at 95% confidence level were subjected to data for statistical analysis.

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INTRODUCTION

The treatment of metastatic non-small cell lung cancer (NSCLC) has been based on cytotoxic chemotherapy. Immunotherapy and tyrosine kinase inhibitors (TKIs) have increased the therapeutic opportunities. Anaplastic lymphoma kinase (ALK) rearrangement is present in approximately 5% of metastatic NSCLC. ALK-positive patients benefit from TKIs, such as Alectinib (AT), a specific second-generation ALK TKI. AT has shown a progression-free survival of 34.8 months compared with 10.9 months with crizotinib, the historical first-line treatment for ALK-positive patients (stratified hazard ratio=0.43; 95% confidence interval: 0.32–0.58). Oral administration of TKIs may not be feasible in certain medical situations such as respiratory failure or swallowing difficulties due to carcinomatous meningitis or mediastinal lymph node involvement. Enteral administration of TKIs may thus be the only available resource (1-3). Furthermore, AT is an oral, highly potent ALK inhibitor that has recently been approved in the European Union (EU) as first-line treatment for adult patients with advanced ALK-positive NSCLC and adult patients with advanced ALK-positive NSCLC previously treated with crizotinib. AT has also been approved in the United States of America (USA) for the treatment of ALK-positive, metastatic NSCLC. In the USA, AT is indicated for the treatment of adults with ALK-positive metastatic NSCLC. The recommended dosage for AT in the EU and USA is 600 mg twice daily. Well-designed phase III studies in patients with ALK-positive NSCLC showed that during up to ≈ 19 months follow-up, progression-free survival (PFS) was significantly improved with AT relative to crizotinib as first-line therapy, and relative to chemotherapy in patients previously treated with crizotinib and platinum-doublet chemotherapy (3-6). The objective of this work was to develop a simple liquid chromatography method, which could serve as an assay method for AT in marketed dosage forms. A survey of the literature revealed the availability of few analytical methods for the quantitative determination of AT alone or in combination with other drugs in biological fluids and marketed formulations. The reported methods were mainly based on liquid chromatographic estimation using UV-VIS, fluorescence, electrochemical, or mass spectrometry detectors (7-14). Hence, an attempt was made to develop a simple, precise, and accurate method for the estimation of AT in pharmaceutical dosage form. The manuscript describes the development and validation of an isocratic reversed-phase HPLC method for the assay of AT as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol and acetonitrile were procured from Thermo Fisher Scientific India Pvt. Ltd. New Delhi, India. Ammonium acetate was procured from Central Drug House (P) Limited, New Delhi, India. Mili Q water was used throughout the study. Other chemicals used in this study were of analytical or HPLC grade.

Instrumentation

The analysis was carried out on Waters Alliance e-2695 separating module (Waters Co., MA, USA) using photodiode array detector (waters 2998) with autosampler and column oven. The instrument was controlled by Empower software (version 6.00.00.00) installed with equipment for data collection and acquisition. C18 HPLC column (250 mm × 4.6 mm i.d., particle size 5 µ), eluted with mobile phase at the flow rate of 1.1 $mLmin^{-1}$ was used.

Chromatographic Conditions

The mobile phase consisted of buffer and acetonitrile (55:45 v/v). The buffer used in the mobile phase contained 50mM ammonium acetate and its pH was adjusted to 5.8±0.02, filtered through 0.45 µm nylon filter and degassed in ultrasonic bath prior to use. Measurements were made with an injection volume 20 µL and UV detection at 292nm. All analyses were performed at ambient temperature.

Standard and Sample Solutions Preparation

Preparation of Standard Stock Solution

The standard solution was prepared by dissolving the drug in the solvent and diluting to the desired concentration by mobile phase as a solvent system. Accurately weighed 100mg of AT was transferred into a 100 mL volumetric flask and dissolved in the mobile phase. A standard solution was prepared from the stock solution by transferring 10mL of stock solution to a 100mL volumetric flask and diluting with mobile phase to get a solution of 100 μgmL^{-1} of AT. The solution was further diluted with mobile phase to obtain the desired concentration (15-18).

Preparation of Sample Solutions

The method was used for quantitation of AT in the marketed formulation (Alesensa). For sample preparation, mobile phase was used as a solvent. Powder equivalent to 50mg of AT was transferred in to 50mL volumetric flask and dissolved in 20mL of the mobile phase. Volume was made up to the mark. The solution was ultrasonicated for 25 min and filtered through a 0.45 µm membrane filter. The solution was further diluted with mobile phase to obtain desired concentration and was subjected to HPLC analysis as described earlier. From the peak area of AT the amount of drugs in samples was computed (15-18).

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability parameters by the ICH guidelines Q2 (R1). To assess the linearity and range of the developed method, seven different mix standard concentrations (10, 20, 30, 40, 50, 60 and 70 μgmL^{-1}) of AT were prepared. The analyses were performed in triplicate. The peak area values were plotted against corresponding concentrations. The accuracy and precision were measured by performing the assay of samples (spiked placebos) prepared at three concentration levels of 50%, 100% and 150% of the standard concentration, with 3 replicates for each concentration. The % recovery and % relative standard deviation (RSD) were calculated for each of the replicate samples. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated based on the standard deviation of the response (σ) and slope approach as defined in ICH guidelines. The LOD was calculated using the formula 3.3*σ/slope, and the LOQ was calculated using the formula 10*σ/slope. The robustness of the method was investigated under a variety of conditions including flow rate, pH and percentage of solvent in the mobile phase (18-22).

RESULTS AND DISCUSSION

In this work an LC method for the determination of AT in bulk drug and pharmaceutical formulations with UV detection was developed and validated as per ICH guidelines for analytical method validation (18-22).

Method Development

The main objective of this work was to develop an HPLC method for the determination of AT within a short run time below 5 min and symmetry between 0.80 and 1.20. The stationary and mobile phases play an important role in theoretical plates, peak shape, symmetry and resolution. To obtain symmetrical peaks with better resolution and peak purity, various chromatographic conditions were investigated and optimized for the determination of AT; such as mobile phases with different compositions, pH and stationary phases with different packing material, etc. Finally, the mobile phase containing buffer (50mM ammonium acetate) and acetonitrile in the ratio of 55:45 v/v (pH 5.8±0.02) was selected and found to be optimal with more theoretical plates (≥21536) and short retention time (4.11, below 5 min). Based on the optimal mobile phase, a highly symmetrical and sharp characteristic peak of AT was further obtained on C18 Column (250 mm × 4.6 mm i.d., particle size 5 µ) with 1.1 $mLmin^{-1}$ flow rate. A typical HPLC chromatogram of a standard solution of AT is given in Figure 1 (15-19).

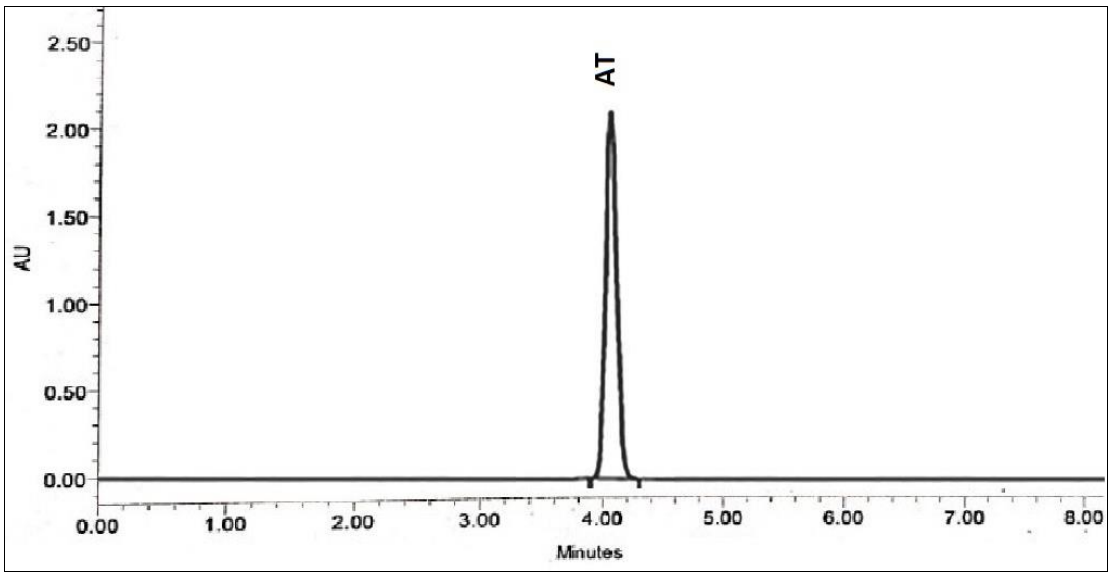

Figure 1. HPLC chromatogram of a standard solution of AT

Method Validation

An optimized method must be validated before actual use. System suitability testing was performed as per ICH guidelines for analytical method validation, Q2 (R1). The validation studies were performed as prescribed in the following sections. Linear regression data demonstrated an excellent relationship over a concentration range of 10-70 μgmL^{-1} for AT. The linear regression equation for AT was found to be y = 12913x – 10933. The regression

coefficient value (r^2) was found to be 0.9994 indicating a high degree of linearity. The linearity curve of AT is given in Figure 2 and linearity parameters for the AT are given in Table 1. The specificity studies depicted the complete absence of any other excipients as no peak was reported during the retention time of AT. Standard deviation and slope-based method was adopted for determining the LOD and LOQ which was respectively found to be 0.92 and 2.9 μgmL^{-1} for AT. The values indicate that the method is sensitive. The lower values of % RSD 0.95 and 1.44 for intra-day and inter-day precisions respectively (Table 2) indicate that the method is precise. Recovery study was carried out using the standard addition method at three different levels of 50%, 100% and 150%. The average % recoveries for AT in marketed formulation were found to be between 99.62 and 100.18 (Table 3). The results revealed that there was no interference. The developed method was successfully applied to analyze AT in marketed formulation. The amounts recovered were expressed as a percentage of the label claim. The average percentage of drug contents of formulation obtained by the proposed method for AT was noted to be 99.88%. These values comply with the official specifications (18-22).

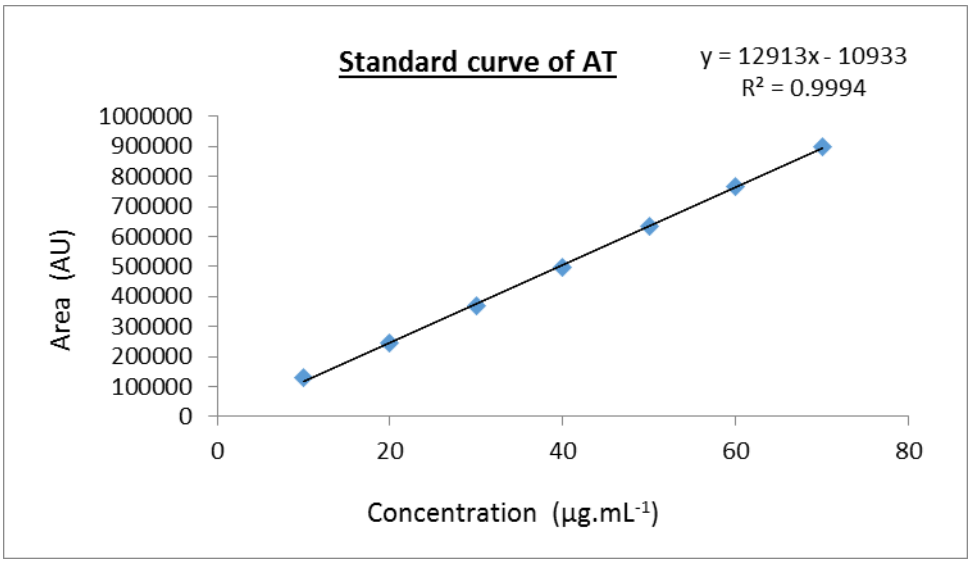

Figure 2. Standard curve of AT

Table 1. Linearity parameters for AT

Linearity Parameter	AT
Range	10-75 µgmL^{-1}
Slope	12913
Intercept	10933
Regression coefficient (r^2)	0.9994
f-test	1.51 (5.47)[a]
t-test	0.48 (3.93)[a]

Table 2. Statistical treatment of the precision data

Parameter	AT
Intra-day or Repeatability (%RSD)	0.95
Inter day (%RSD)	1.44
f-test	3.99 (6.39)[a]
t-test	1.71 (2.74)[a]

Table 3: Percent recovery data AT

% simulated dosage nominal	% Mean (n=3)	RSD (%)
50	99.83	0.66
100	100.18	0.79
150	99.62	0.41

System Suitability Parameters

For system suitability parameters, six replicates of the standard solution were injected. All critical parameters met the acceptance criteria on all days. Parameters such as resolution, capacity factor, tailing factor, theoretical plate, retention volume, and asymmetry factor of the peaks were calculated. The results are shown in Table 4 (18-22).

Table 4. System suitability data for AT

Parameters	ET
Retention time (min)	4.11
Injection precision RSD (%)	0.51
Resolution	-
Tailing factor	1.08
Theoretical plates	23332
Capacity factor	0.72
Retention volume	4.52

CONCLUSION

A simple isocratic reversed-phase HPLC method for the estimation of AT was developed and validated as per ICH guidelines. Validation experiments proved that the HPLC method is linear in the proposed working range as well as accurate, precise and specific. The good recovery percentage of the marketed formulation suggests that the excipients have no interference in the determination. The RSD was also less than 2% showing a high degree of precision of the method. The proposed method was also found to be robust with respect to flow rate and composition of the mobile phase. In addition, simple isocratic elution and easy extraction procedures offered rapid and cost-effective analysis of the drugs. *f*-test and *t*-test were applied to the data at 95% confidence level, and no statistically significant difference was observed. The proposed method can be used for routine analysis of AT in marketed dosage forms and in quality control in bulk manufacturing as well.

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