HPTLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF ALECTINIB HYDROCHLORIDE IN BULK POWDER AND CAPSULE PREPARATIONS

Shaileshkumar K. Koradia[a]*, Rushik P. Thakkar[a], Ashok N. Mahajan[a], Falgun A. Mehta[a], Priyal R. Patel[a] and Digvijaysinh G. Rana[a]

(Received 20 May 2022) (Accepted 20 June 2023)

ABSTRACT

The high performance thin layer chromatography method described here enables simple, accurate and precise estimation of alectinib hydrochloride content in capsule formulation. To achieve adequate separation, G60 - F254 pre-coated silica gel sheets of aluminium were used as the stationary phase. A mobile phase comprising a solvent mixture of methanol and water in a ratio of 80:20 (V/V) was utilized with a chamber saturation duration of 30 minutes. The retardation factor value was found to be 0.47 at the detection wavelength 341 nm. The method's linearity was confirmed across a concentration range from 600 - 2100 ng band^{-1}, exhibiting a high correlation coefficient of 0.9992. The accuracy range was observed between 98.50 % – 100.45 %. The lowest detection limits of drug were observed to be 17.49 ng band^{-1} and lowest quantitation limits of drug were found to be 53.01 ng band^{-1}. This developed methodology was effectively employed for the quantification of alectinib hydrochloride in capsule preparations.

Keywords: Alectinib hydrochloride, HPTLC, anti-neoplastic drug, analytical method validation, capsule formulation

INTRODUCTION

Alectinib hydrochloride is an orally available tyrosine kinase inhibitor with anticancer properties. It is chemically known as 9-ethyl-6,6-dimethyl-8-[4-(morpholin-4-yl) piperidin-1-yl]-11-oxo-6,11-dihydro-*5H*-benzo[*b*] carbazole-3-carbonitrile hydrochloride with a molecular weight of 519.08 Dalton, and its empirical formula is $C_{30}H_{34}N_4O_2$•HCl[1-2]. Its chemical structure is shown in Fig. 1. U.S. Food and Drug Administration (USFDA) approved alectinib hydrochloride as a therapeutic option for patients diagnosed with anaplastic lymphoma kinase-positive metastatic non-small cell lung cancer (NSCLC) in 2017. It blocks the action of abnormal proteins that signal cancer cells to proliferate[3]. Alectinib hydrochloride is not officially listed in Indian Pharmacopoeia, United States Pharmacopoeia, or British Pharmacopoeia. A literature review indicates the determination of alectinib hydrochloride in pharmaceuticals and biological fluids using the UPLC-MS/MS[4-5], UPLC-PAD[6], and RP-HPLC[7-8] methods. Based on our current understanding, there has been no reported HPTLC method for the assessment of alectinib hydrochloride. The most important advantage of the HPTLC method is that, unlike HPLC, it uses a small amount of mobile phase to analyze multiple samples simultaneously, reducing analysis time, sample purification, and analysis costs per sample[9].

MATERIALS AND METHODS

Chemicals

Alectinib hydrochloride was procured as a complimentary sample from Sun Pharmaceutical Industries Ltd., Vadodara, Gujarat. Capsule formulations with a strength of 150 mg were prepared in the laboratory using common excipients. Analytical-grade methanol was purchased from S. D. Fine Chemicals, Mumbai, India

Instrumentation and software

HPTLC analysis of samples was performed on HPTLC CAMAG Linomat using WinCATS software version 1.4.8. TLC plates pre-coated with G60 – F254 silica gel on aluminum sheets with dimensions of 10 cm × 10 cm and thickness of 200 µm, manufactured by Merck, Germany was used. A CAMAG twin trough glass chamber was used

[a] Department of Pharmaceutical Chemistry & Quality Assurance, Krishna School of Pharmacy & Research, Drs. Kiran & Pallavi Patel Global University, Krishna Educational Campus, Vadodara – Mumbai NH # 8, Varnama, Vadodara – 391 243, Gujarat, India

*For Correspondence: E-mail: shaileshkoradia.ksp@kpgu.ac.in

https://doi.org/10.53879/id.60.10.13519

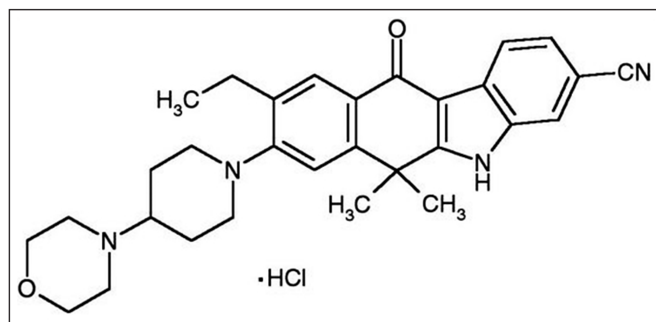

Fig. 1: Chemical structure of alectinib hydrochloride

to develop the TLC plate. The sample received pressurized nitrogen gas through a 100 µL syringe attached to a CAMAG Linomat 5 sample applicator. Subsequently, the band was observed under a CAMAG UV cabinet at the wavelength of 254 nm. The band was visualized at 254 nm in a CAMAG UV cabinet. An electronic scale AX200 manufactured by Shimadzu Ltd., Japan, was used for this project.

HPTLC METHOD DEVELOPMENT

Preparation of reference solutions

An exact weight of 100 mg of alectinib hydrochloride was placed in a 100 mL glass stoppered flask. The drug was dissolved and then further diluted with methanol to achieve a final solution with a concentration of 1000 µg mL^{-1}. Subsequently, this resultant solution was further diluted with methanol to create a working reference solution with a concentration of 100 µg mL^{-1}.

Stationary phase

TLC plates pre-coated with G60 – F254 silica gel on aluminum sheets with dimensions of 10 cm x 10 cm and thickness of 200 µm, manufactured by Merck, Germany was used. The TLC plates were pre-washed with methanol and pre-conditioned prior to use.

Mobile phase

The mobile phase was prepared by appropriate mixing of solvent methanol and water in a ratio of 80:20(V/V), The chamber was saturated with the mobile phase for 30 minutes.

Chromatographic conditions

The sample applicator used was a CAMAG Linomat 5. It placed samples 6 mm in size and 15 mm from the bottom of the plate under nitrogen gas pressure. Chromatograms were obtained using methanol:water (80:20; V/V) mobile phase in a twin trough chamber saturated with 10 mL of mobile phase for 30 minutes. After TLC plate development, densitometry scanning was performed using WinCATS software. A deuterium lamp served as the radiation source, and scanning was conducted at a wavelength of 341 nm.

Preparation of sample solution

20 capsules with a label claim of 150 mg of alectinib hydrochloride were precisely weighed after the powder from the shells had been completely removed. Exactly weighed capsule powder containing 100 mg of alectinib hydrochloride was transfered into a 100 mL glass stoppered flask. To obtain 1000 µg mL^{-1} solution, it was dissolved in methanol and diluted to 100 mL. The stock solution was appropriately diluted with methanol to achieve a 100 µg mL^{-1} concentration of the solution, and subsequently, filtered through Whatman filter paper No. 41.

Validation of HPTLC method

The International Council for Harmonisation (ICH) guideline recommendations Q2-R1 were successfully followed in the validation of the developed HPTLC method for alectinib hydrochloride determination in capsule formulation. The factors investigated included linearity and range, accuracy, precision of the method, lowest detection and quantitation limits, specificity and robustness[10].

Linearity and range

The method's linearity was tested at 6 different concentration levels of alectinib hydrochloride, ranging from 600 to 2100 ng band^{-1}. The linearity curve was constructed by graphing the response factor as the peak area versus the drug concentration (n=3) and slope, intercept, correlation coefficient and equation of regression was calculated. The lower and upper ranges of drug concentration were 600 ng band^{-1} and 2100 ng band^{-1}, respectively.

Accuracy

Recovery studies were used to test the accuracy of the method. In accordance with ICH recommendations, the standard solutions of alectinib hydrochloride were spiked (300, 600, and 900 ng band^{-1}) into a pre-analyzed sample solution of 600 ng band^{-1}.

Precision

The repeatability of the method was evaluated using standard solution of 600 ng band^{-1} of alectinib hydrochloride, which was injected six times (n = 6) and peak areas of injected solutions were measured and

% RSD was calculated. Intraday precision of proposed method was evaluated by carrying out triplicates of various concentrations such as lowest 600 ng band^{-1}, medium 1500 ng band^{-1} and highest 2100 ng band^{-1} on the same day and response factor (peak area) determined was reported as % RSD. The interday precision of the method was checked on three consecutive days using the mentioned concentrations of drug and % RSD was calculated.

Specificity

Analyzing the peak purity of drug in reference and test solutions allowed analysts to assess the specificity of method. The R_f values and peak purity spectra of the drug were examined in relation to a standard sample following the chromatographic development of the alectinib hydrochloride band. Furthermore, the sample spectra were examined at three distinct levels, namely, peak leading edge, peak maxima, and peak tailing edge, to determine the peak purity of the drug and to verify whether any interference was present.

Detection limits (LOD) and quantitation limits (LOQ)

According to the equation described in the guidelines, the parameters LOD and LOQ for the suggested method were established.

$$LOD = 3.3 \times \sigma/S,$$

$$LOQ = 10 \times \sigma/S;$$

where, σ = Standard deviation of y intercepts, S = slope of calibration curve.

Robustness

Minor adjustments were made to three chromatographic conditions, specifically the mobile phase composition, chamber saturation time, and detection wavelength, to observe the resulting effects. This was done to demonstrate the robustness of the suggested HPTLC method. In triplicate, samples were placed on TLC plates, which were then processed using an optimized mobile phase. Plates were scanned under optimized chromatographic conditions, and the % RSD for slight variations in each condition was computed.

System suitability parameters

The chromatographic system must pass the limits of system suitability parameters before performing the analysis of sample. Standard solutions of alectinib hydrochloride (600 – 2100 ng band^{-1}) were analyzed under optimized chromatographic conditions for calculating tailing factor and purity index.

Analysis of in-house capsule formulation

The content of alectinib hydrochloride was determined by analyzing the in-house capsule formulation. It was estimated by applying the aliquot of 6 µL from 100 µg mL^{-1} sample solution of alectinib hydrochloride on TLC plate. The TLC plate was developed using optimized chromatographic parameters. Regression line equation was used to determine the alectinib hydrochloride drug content in the formulation.

RESULTS AND DISCUSSION

HPTLC method development

Mobile phase for HPTLC method was optimized based on trial and error. The densitogram of alectinib hydrochloride obtained from mobile phase methanol : water (80:20 V/V) is shown in Fig. 2. This mobile phase gave an appropriate peak shape, with R_f value of 0.47 and asymmetric factor of 1.05. As a result, this mobile phase was chosen as an optimal mobile phase. The detection wavelength for the analysis was chosen as 341 nm, since alectinib hydrochloride absorbed significantly at that wavelength.

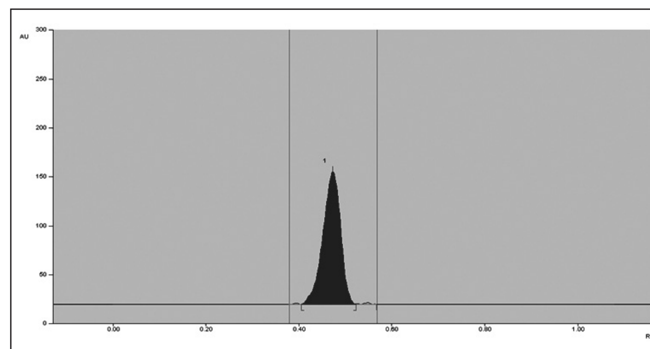

Fig. 2: HPTLC densitogram of standard alectinib hydrochloride (600 ng band^{-1}, R_f = 0.47)

VALIDATION OF HPTLC METHOD

Linearity and range

The alectinib hydrochloride calibration curve was plotted in the 600–2100 ng band^{-1} range with a satisfactory correlation co-efficient (r^2) of 0.9992. Fig. 3 depicts three-dimensional overlain densitograms of 600 to 2100 ng band^{-1}. Table I illustrates the regression analysis of the calibration curve for the HPTLC method.

Table I: Regression analysis of calibration curve for alectinib hydrochloride

Parameter	Result
Concentration range (ng band^{-1})	600 - 2100 ng band^{-1}
Regression co-efficient (r^2)	0.9992
Regression equation (y = mx + c)	y=3.1846x+2397.3
Slope of regression equation	3.1846
Standard deviation of slope	0.0026
Intercept of regression equation	2397.3
Standard deviation of intercept	16.880

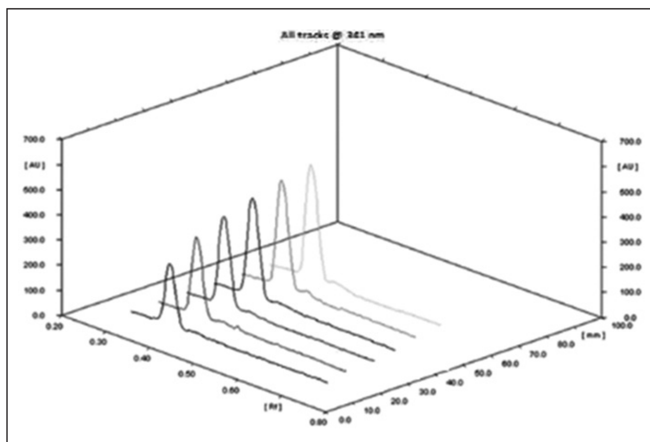

Fig. 3: 3D densitogram showing linearity of alectinib hydrochloride (600 – 21000 ng band^{-1})

Accuracy

Recovery studies of alectinib hydrochloride from the capsule formulation at three distinct and different levels (80 %, 100 %, and 120 %) of standard addition were used to determine the accuracy of the method. The percent recovery was determined to be between 98.50 % and 100.45 %, proving the accuracy of the established HPTLC method.

Precision

The system precision was determined by analyzing peak areas and retardation factor of injected sample solution. The precision value in terms of % RSD was found to be 0.61 % for peak area and 0.44 % for retardation factor. Hence, the % RSD less than 1.0 % indicates that the method is repeatable. Additionally, % RSD values were determined to be 0.65 % - 1.32 % and 1.09 % - 1.76 %, respectively, for the intra-day precision and inter-day precision studies.

The values confirm the precision of the developed HPTLC method.

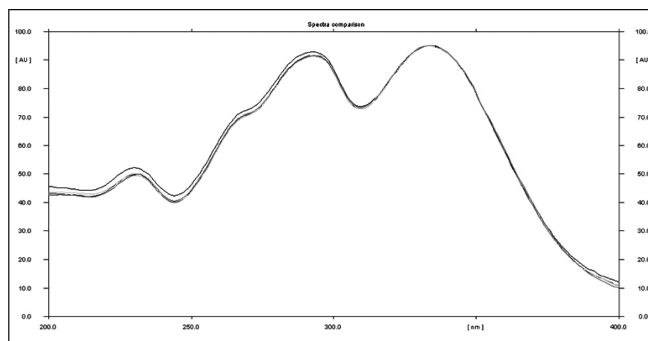

Fig. 4: Overlain spectra of formulation with standard alectinib hydrochloride showing peak purity

Table II: Validation parameters for alectinib hydrochloride

Parameter	Result
Detection wavelength	341.0 nm
Linearity	600 - 2100 ng band^{-1}
Detection limits	17.49 ng band^{-1}
Quantitation limits	53.01 ng band^{-1}
Precision (% RSD) • Repeatability (Peak area) • Repeatability (Retardation factor) • Intraday • Inter-day	 0.61 % 0.44 % 0.65 % – 1.32 % 1.09 % – 1.76 %
Accuracy (% Recovery)	98.50 % - 100.45 %
Robustness	Robust
Specificity	Specific

Specificity

The overlay spectra of alectinib hydrochloride in sample and standard were compared to assess the specificity and peak purity of the compound. Fig. 4 shows the overlapping spectra of alectinib hydrochloride in standard solution and sample solution.

Detection limits (LOD) and quantitation limits (LOQ)

LOD and LOQ values of drug emerged to be 17.49 ng band^{-1} and 53.01 ng band^{-1}, respectively, for the suggested HPTLC method. It indicates that the proposed method is sensitive enough for the detection and quantitation of alectinib hydrochloride effectively.

Robustness

To evaluate the method's robustness, deliberate minor adjustments were introduced to the chromatographic parameters, including modifications to the mobile phase composition, chamber saturation time and detection wavelength. The % RSD for peak area ranged from 0.51 % to 1.17 %, while for the retardation factor, it ranged from 0.76 % to 0.92 %. The absence of significant fluctuations in both peak area and retardation factor affirmed the robustness of the method.

System suitability parameters

During the assessment of the method's system suitability, it was observed that the tailing factor was below 2, and the purity index was close to 1. This indicates that the equipment, procedure, and samples are all essential components of the system, and the sample can be evaluated accordingly using the established method. Table II summarizes the validation parameter results.

Analysis of in-house capsule formulation

The newly developed HPTLC method was tested by assessing an in-house capsule formulation of alectinib hydrochloride. The content of the drug fell within the range of 99.48 % to 100.54 %, demonstrating adherence to the specification limits. Thus, the established HPTLC technique is suitable for regular quality control assessments of alectinib hydrochloride.

CONCLUSION

The proposed HPTLC method is superior to other chromatographic methods, according to the chromatographic process, satisfying results of many parameters, and drug content. The suggested HPTLC method was effectively applied for analysis of drug from capsule dosage form and could be routinely used in the quality control laboratory for the determination of alectinib hydrochloride.

REFERENCES

1. Collins L.G., Haines C., Perkel R. and Enck R.E.: Lung cancer: Diagnosis and management, **Am. Fam. Physician,** 2007, 75(1), 56-63.
2. Wong S.Y.: Apoptosis in cancer: from pathogenesis to treatment, **J. Exp. Clin. Cancer Res.,** 2011, 30(1), 1-14.
3. Karachaliou N., Bruno M. F., Bracht J. W. P. and Rosell R.: Profile of alectinib for the treatment of alk-positive non-small cell lung cancer (NSCLC): Patient selection and perspectives, **Oncol. Targets Ther.,** 2019, 12(2), 4567-4575.
4. Hadir M. M., Aliyah A., Nourah Z. A., Shereen M. S. and Ashwaq A. A.: Development and validation of UPLC-MS/MS method for the simultaneous quantification of anaplastic lymphoma kinase inhibitors, alectinib, ceritinib, and crizotinib in Wistar rat plasma with application to bromelain-induced pharmacokinetic interaction **J. Pharm. Biomed. Anal.,** 2021, 204, 114276.
5. Huang X. X., Li Y. X., Li X. Y., Hu X. X., Tang P. F. and Hu G. X.: An UPLC-MS/MS method for the quantitation of alectinib in rat plasma, **J. Pharm. Biomed. Anal.,** 2017, 132(4), 227-231.
6. Kumar H. K. S., Sahu S. K. and Debata J.: A novel ultra performance liquid chromatography-PDA method development and validation for alectinib in bulk and its application to tablet dosage form, **Int. J. Pharm. Investig.,** 2020, 10(4), 537-541.
7. Pavani B. and Mounica P.: RP-HPLC method development and validation for estimation of Alectinib in bulk and pharmaceutical dosage form, **Int. J. Pharm. Anal. Res.,** 2019, 8(3), 293-300.
8. Prashanthi Y., Rao T. N. and Srinivas Y.: Method development and validation of alectinib drug by RP-HPLC in bulk and pharmaceutical dosage form, **Asian J. Pharm. Anal.,** 2018, 8(4), 186-90.
9. Sivakumar T., Manavalan R. and Valliappan K.: Global optimization using derringer's desirability function: Enantio selective determination of ketoprofen in formulation and biological matrices, **Acta Chromatogr.,** 2007, 19, 29-47.
10. ICH Guideline [Validation of Analytical Procedures: Methodology (Q2R1)], International Conference on Harmonization, Food and Drug Administration, USA, 1996 & 2005.

For Advertising in the Classified Columns and also for series: advertisements please contact

Publications Department

Tel.: 022-249704304 / 66626901

E-mail: melvin@idmaindia.com/ geeta@idmaindia.com

Website: www.idma-assn.org, www.indiandrugsonline.org