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Review Article

FORCED DEGRADATION STUDIES AND REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD VALIDATION FOR THE DETERMINATION OF CERITINIB IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple stability indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of ceritinib present in pharmaceutical dosage forms. The reported RP-HPLC method utilizes a BDS C18 Column (150 mm × 4.6 mm, 5 µm) in an isocratic separation mode. The mobile phase consists of 0.01 N potassium dihydrogen orthophosphate (KH_2PO_4) buffer and acetonitrile in the ratio 55:45 (%v/v). The pH was adjusted to 4.5 with dilute orthophosphoric acid and flow rate was maintained at 1.0 ml/minutes and elute was monitored by using the ultraviolet detector at 320 nm wavelength. The retention time of ceritinib was 2.539 minutes. The method was validated as per ICH guidelines with respect to specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability parameters. The LOD and LOQ values were 0.02 and 0.06 ppm, respectively. The linearity of the drug was in the range of 25-150 µg/ml with correlation coefficient of 0.999. The percentage recoveries of the ceritinib drug was ranged from 98.46% to 99.97%. The optimized method was proved to be specific, robust, and accurate for the quality control of ceritinib in pharmaceutical preparations. The stability of the drug was examined under different stress conditions forcibly. The method was successfully applied for routine analysis of ceritinib in tablet dosage form.

Keywords: Ceritinib, Reversed-phase high-performance liquid chromatography, Stability validation, Ultraviolet detection.

INTRODUCTION

Ceritinib, 5-Chloro-N4-[2-[(1-methylethyl)sulfonyl]phenyl]-N2-[5-methyl-2-(1-methylethoxy)-4-(4-piperidinyl)phenyl]2,4-pyrimidine diamine, is an anaplastic lymphoma kinase (ALK) inhibitor which induces complete tumor regression in a xenograft model of EML4-ALK-positive lung cancer. The alternative names of ceritinib are LDK 378, NVP-LDK 378, Zykadia™. Ceritinib is a highly selective inhibitor of an important cancer target, ALK [1]. Ceritinib a recently approved drug by Food and Drug Administration is used for the treatment of late-stage (metastatic) non-small cell lung cancer [2]. The recommended dosage of ceritinib is 750 mg administered orally once daily on an empty stomach [3]. An ultrafast, sensitive, selective, and robust LDTD-APCI-MS/MS method was developed for the quantification of ceritinib in human plasma [4]. A stability indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for estimation of ceritinib was reported [5]. Since, there is only one HPLC method reported in the literature for the estimation of ceritinib in pharmaceutical dosage forms, there is a need to develop quantitative methods under different conditions to achieve improvement in sensitivity, selectivity, etc., Hence, the authors have attempted to develop a stability indicating method with different experimental conditions for the estimation of ceritinib. The chemical structure of ceritinib was shown in Fig. 1.

EXPERIMENTAL

Chemicals and reagents

The reference sample and branded formulation of ceritinib was supplied by M/s Spectrum labs, Hyderabad, Telangana state, India. HPLC grade acetonitrile (ACN), double distilled water, methanol and hydrogen peroxide were purchased from E Merck Co., Mumbai, India and potassium dihydrogen orthophosphate, orthophosphoric acid AR grade were purchased from SD Fine Chem, Mumbai, India. High pure water was prepared by using Millipore Milli-Q plus purification system. Commercial formulations of Zykadia™ containing ceritinib was purchased from the local market.

HPLC instrumentation

Quantitative determination of ceritinib was performed on waters HPLC model 2695 equipped with ultraviolet (UV) -visible detector using data handling system-waters alliance empower two software. For weighing the samples, the sartorious electronic balance was used. For degassing and mixing of the mobile phase, ultra-sonic bath sonicator was used.

Chromatographic conditions

The chromatographic separations of ceritinib were performed using BDS C_{18} column (150 mm × 4.6 mm, 5 µm) maintained at 30°C temperature, eluted with mobile phase at a flow rate of 1.0 ml/minutes and run time was set for 6 minutes. Separation was carried out using a mobile phase of the buffer (pH=4.5, potassium dihydrogen orthophosphate buffer) and acetonitrile (ACN) taken in the ratio 55:45 (%v/v). A mixture consisted of methanol and water in the ratio 50:50 (%v/v) was used as diluent. Measurements were made with injection volume 10 µL and UV detection was made at a wavelength of 320 nm. The data was analyzed on empower 2 software version. Before analysis, the mobile phase was degassed by use of a sonicator and filtered through a 0.45 µm filter. Sample solutions were also filtered through a 0.45 µm filter.

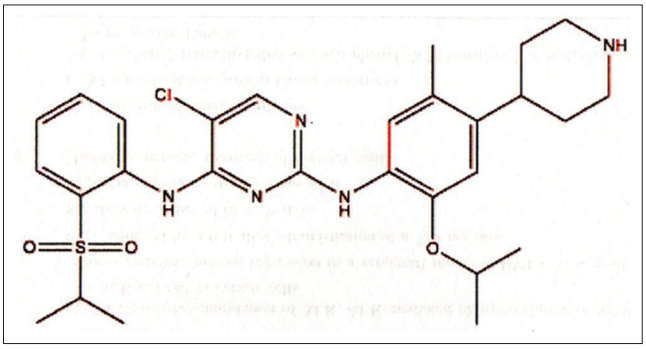

Fig. 1: Structure of ceritinib

Mobile phase

Accurately weighed 1.36 g of potassium dihydrogen orthophosphate in a 1000 ml of the volumetric flask and about 900 ml of milli-Q water was added. The solution was filtered through 5 µ membrane filter and was degassed. A freshly prepared buffer and ACN in a ratio of 55:45% v/v was used as the mobile phase. For preparing the working solution of the ceritinib drug, mobile phase was used as diluent.

METHOD DEVELOPMENT

Preparation of standard stock solution

Accurately weighed and transferred 10 mg of ceritinib working standards into a 10 ml clean dry volumetric flask, 7 ml of diluents were added, sonicated for 30 minutes and made up to the final volume with diluents to prepare a 100 µg/ml standard stock solution. From the above stock solution, 1 ml was pipetted out into a 10 ml volumetric flask and then made up to the final volume with diluents. Standard solutions of ceritinib having concentration in the range of 25-150 µg/ml were prepared by diluting stock solution with mobile phase. The chromatogram of standard solution of ceritinib was given in Fig. 2.

Preparation of sample solution

One tablet of ceritinib sample was weighed and powdered. 150 mg of powdered ceritinib was transferred into 10 ml volumetric flask containing 7 ml of diluents (mixture of methanol and water in 50:50 v/v ratio) and sonicated for 25 minutes, further the volume was made up with diluents and filtered. From the filtered solution, 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluents. Sample ceritinib concentration of 100 µg/ml was obtained.

FORCED DEGRADATION STUDIES

Forced degradation of each drug substance and the drug product was carried out under different stress studies like oxidation, acid, alkaline, thermal, photolytic, and neutral degradation studies.

Peroxide (oxidation) degradation studies

To 1 ml of a stock solution of ceritinib, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 minutes at 60°C. For HPLC study, the resultant solution was diluted to obtain 100 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample (Fig. 3).

Acid degradation studies

To 1 ml of a stock solution of ceritinib, 1 ml of 2 N hydrochloric acid was added and refluxed for 30 minutes at 60°C. The resultant solution was diluted to obtain 100 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 4).

Alkali degradation studies

To 1 ml of a stock solution of ceritinib, 1 ml of 2 N sodium hydroxide was added and refluxed for 30 minutes at 60°C. The resultant solution was diluted to obtain 100 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 5).

Thermal degradation studies

The standard drug solution was placed in an oven at 105°C for 6 hrs to study heat degradation. For HPLC study, the resultant solution

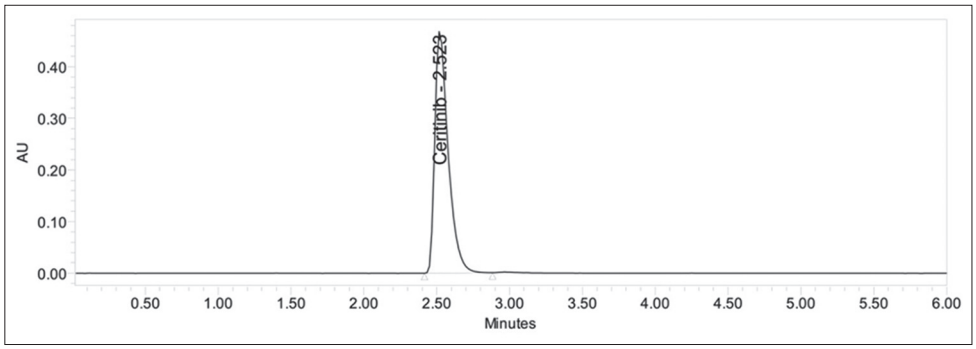

Fig. 2: Standard chromatogram of ceritinib

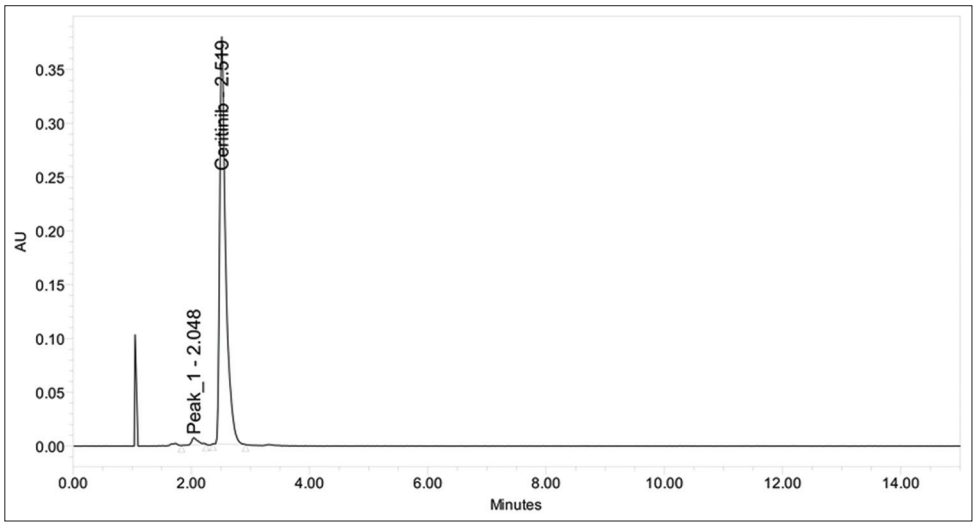

Fig. 3: Chromatogram of peroxide of ceritinib

was diluted to 100 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 6).

Photo stability studies

The photochemical stability of the drug was also studied by exposing the 120 µg/ml solution to UV light by keeping the beaker in UV Chamber

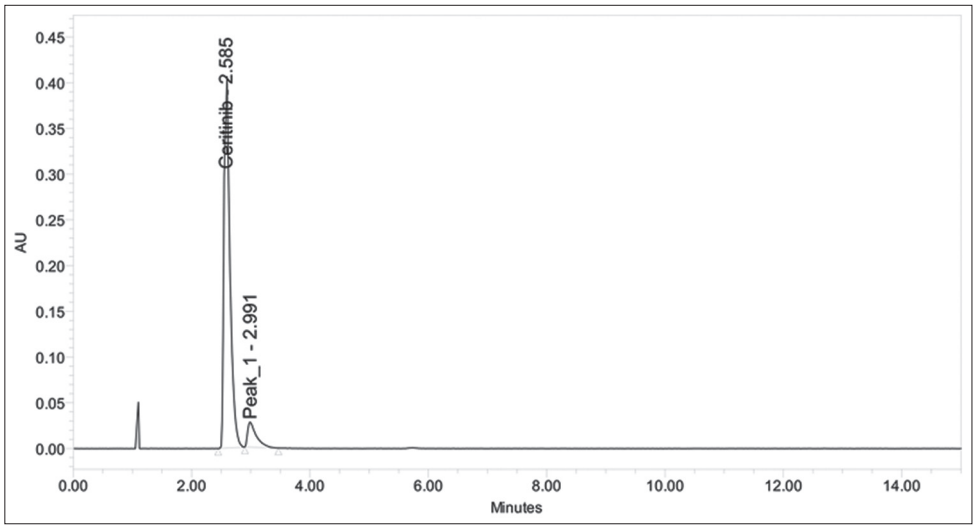

Fig. 4: Chromatogram of acid sample of ceritinib

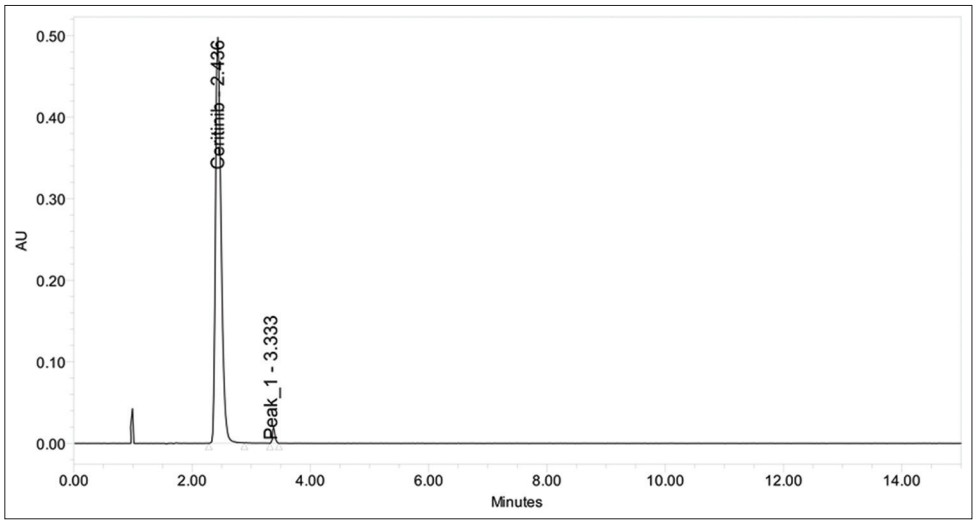

Fig. 5: Chromatogram of alkali sample of ceritinib

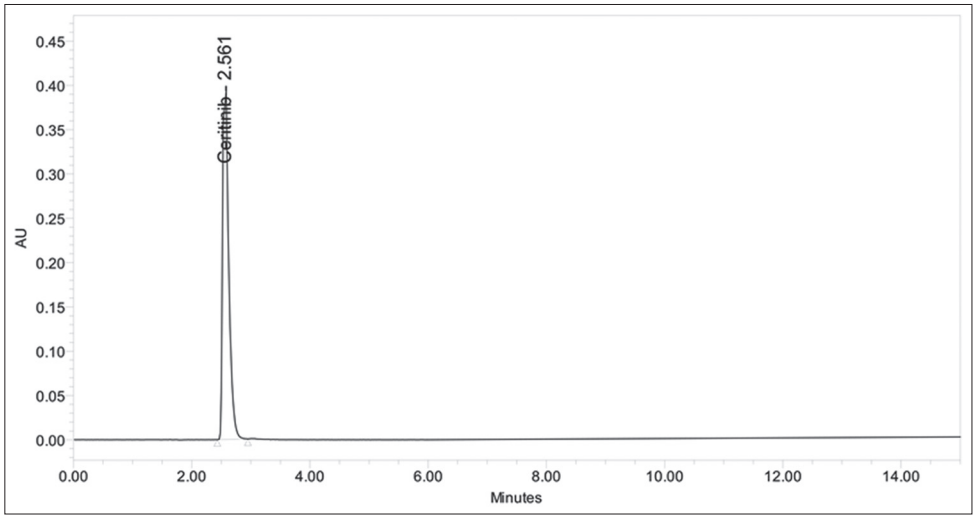

Fig. 6: Chromatogram of thermal sample of ceritinib

for 7 days or 200 W/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 100 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 7).

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 100 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 8).

Results of forced degradation studies

Much degradation was observed in ceritinib samples under stress conditions such as acid, thermal and alkaline studies. Same degradation was observed under stress conditions such as photolytic and aqueous conditions. Least degradation was observed in the presence of peroxide. Table 1 indicates the extent of degradation of ceritinib under various stress conditions. Drug degradation was observed when ceritinib was treated with mild alkali (2 N NaOH for 30 minutes) and a new degradant was eluted at 3.333 minutes. When exposed to sunlight for 7 days, its degradants show a peak at 2.405 minutes, which indicates that ceritinib was sensitive to light. Under oxidation stress, one major

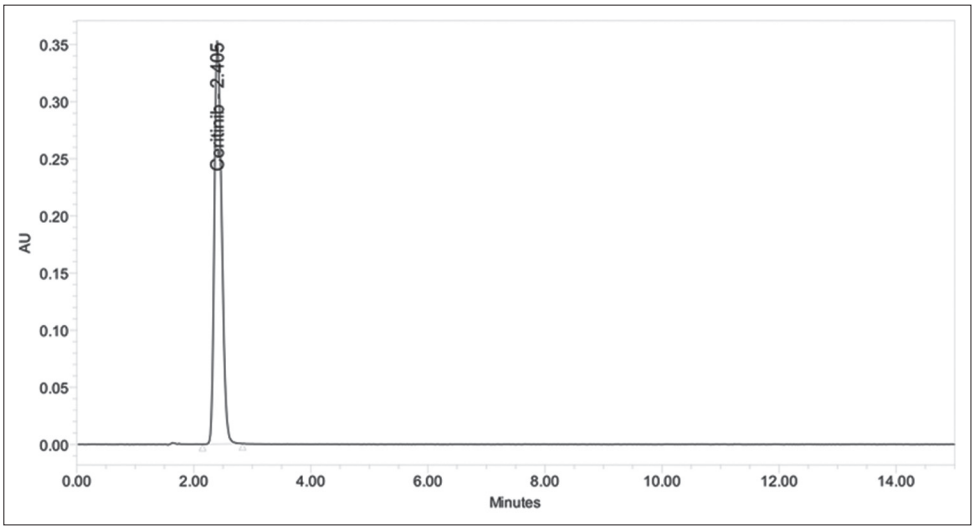

Fig. 7: Chromatogram of ultra violet sample of ceritinib

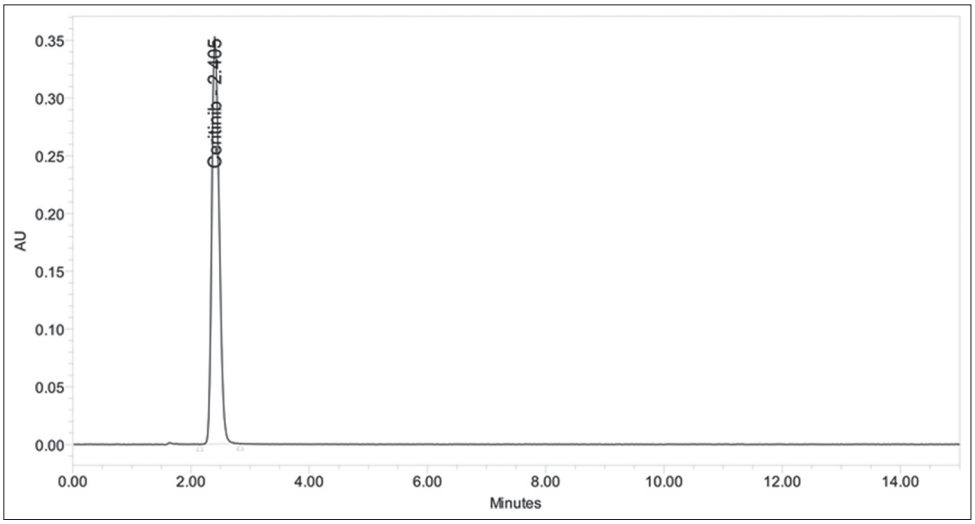

Fig. 8: Chromatogram of water sample of ceritinib

Table 1: Forced degradation data for ceritinib

S. No.	Stress condition	Time (hrs)	Retention time (minutes)	Retention time of additional degradation peak (minutes)	Area	Percentage of active drug after degradation
1	Peroxide	0.5	2.519	2.048	2851604	95.18
2	Acid	0.5	2.585	2.991	2754163	91.92
3	Alkali	0.5	2.436	3.333	2787818	93.05
4	Thermal	6.0	2.561	-	2853698	95.25
5	Photolytic	168.0	2.405	-	2921808	97.52
6	Aqueous	6.0	2.405	-	2961808	98.85

degradant and some unknown degradation products were formed. Assay studies were carried out for stress samples against ceritinib qualified working standard. Assay of ceritinib was unaffected by the presence of other degradants which confirms the stability indicating power of the method.

RESULTS AND DISCUSSION

A reverse phase HPLC method was studied in the present study for the determination of ceritinib tablets in combined dosage form. The column used in this method was BDS C_{18} (150 mm × 4.6 mm,

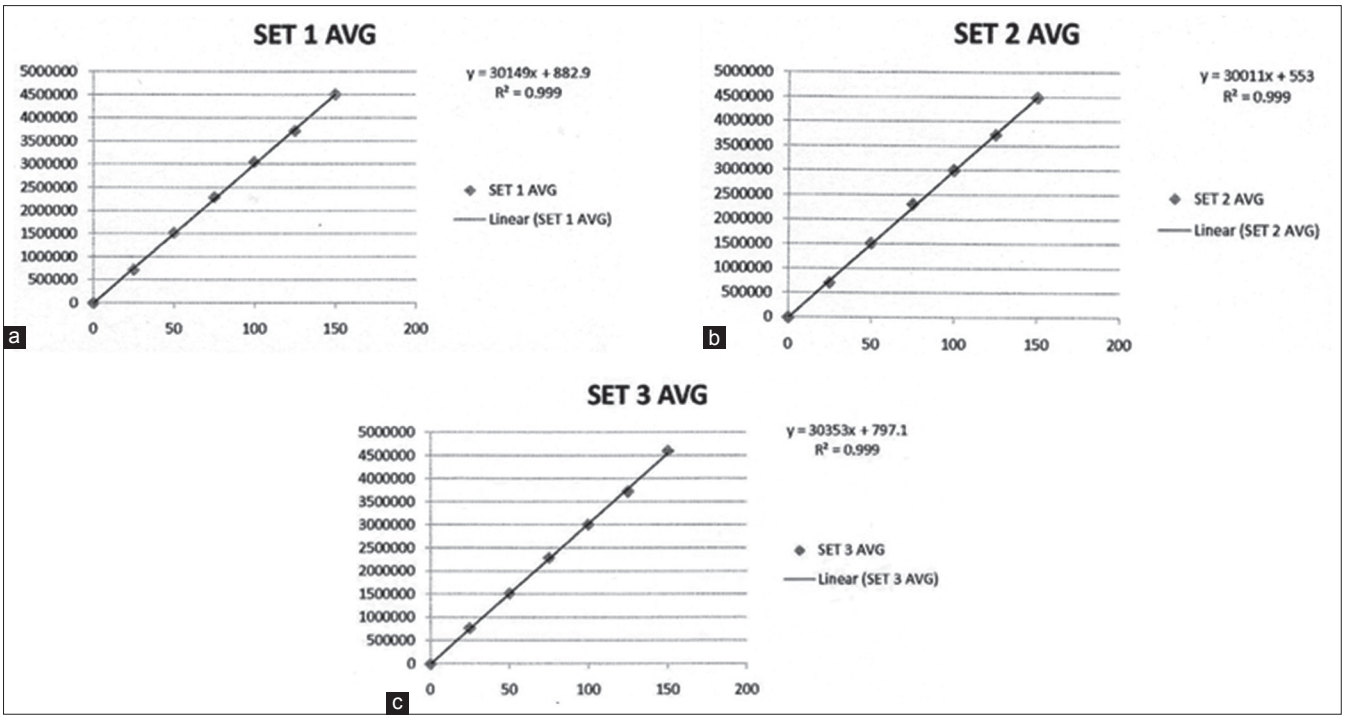

Fig. 9: Calibration curves of ceritinib, (a) Set 1, (b) set 2, (c) set 3

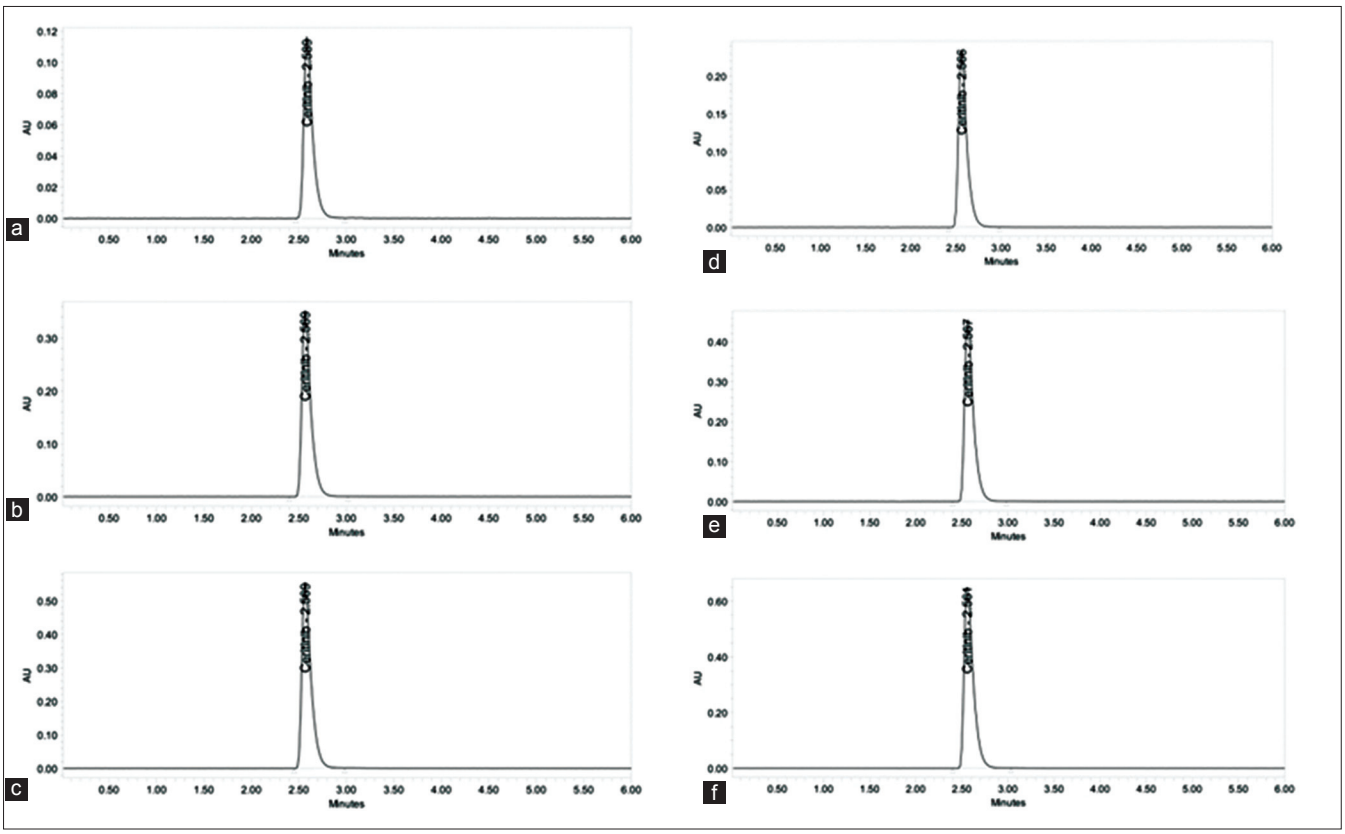

Fig. 10: Linearity chromatograms of ceritinib, (a) 20%, (b) 50%, (c) 70%, (d) 100%, (e) 120%, (f) 150%

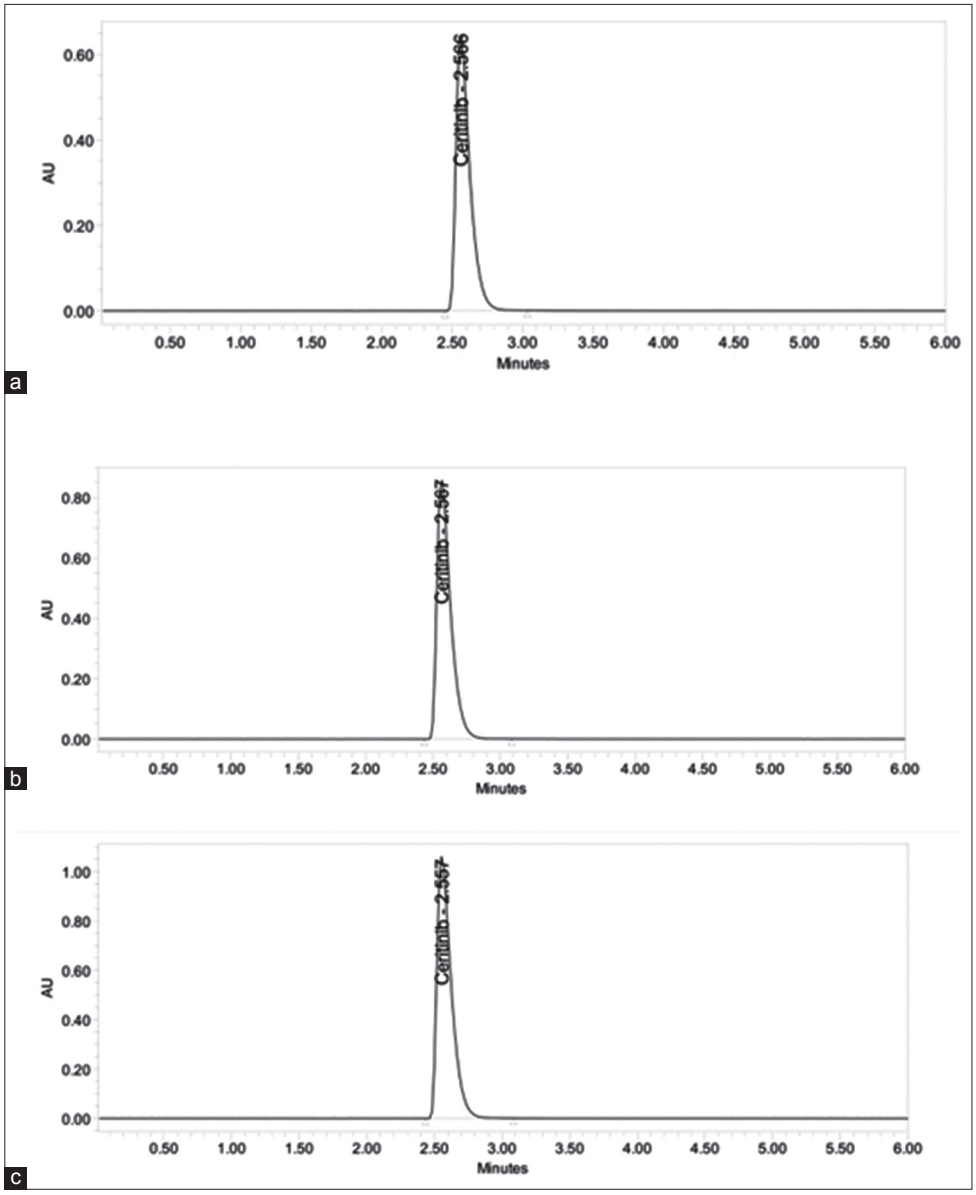

Fig. 11: Accuracy chromatograms of ceritinib, (a) 50%, (b) 100%, (c) 150%

Table 2: Chromatographic conditions of ceritinib

S. No	Method parameters	Method conditions
1	Mobile phase	Buffer and ACN in ratio of 55:45% v/v
2	Column	BDS C_{18}, 150 mm×4.6 mm; 5 µm
3	Detector wave length	320 nm
4	Flow rate	1.0 ml/minutes
5	Injection volume	10 µl
6	Diluent	Methanol and water in 50:50 ratio
7	pH	4.5
8	Column temperature	30°C
9	Run time	6 minutes
10	Retention time	2.539 minutes

ACN: Acetonitrile

Table 3: Linearity studies for ceritinib by proposed method

Concentration (µg/mL)	Area			Average area (μV^2 seconds)
	Set 1	Set 2	Set 3	
25	715173	705381	768661	729738
50	1523154	1517894	1519777	1520275
75	2298188	2310218	2295498	2301301
100	3053698	3005736	3017516	3025650
125	3728358	3730321	3731324	3730001
150	4515764	4489910	4607918	4537864

5 µm) with a flow rate of 1.0 ml/minutes at a wavelength 320 nm and column temperature of 30°C. The mobile phase preparation was done by using buffer: Potassium dihydrogen orthophosphate (pH=4.5). The mobile phase combination was buffer: ACN (55:45 %v/v). The run time was set for 6 minutes. The retention time of ceritinib was 2.539 minutes. The new HPLC method developed and validated for determination of ceritinib in pharmaceutical dosage forms, and assured the satisfactory precision and accuracy, and also determining the lower concentration of drug in its solid combined dosage form by RP-HPLC method. The linearity range for ceritinib was 25-150 µg/ml and the correlation coefficient was found to be 0.999. The percentage relative standard deviation (RSD) obtained for method precision of ceritinib was 0.8. The limit of detection (LOD) and limit of quantification (LOQ) values for ceritinib were 0.02 and 0.06 µg/ml respectively.

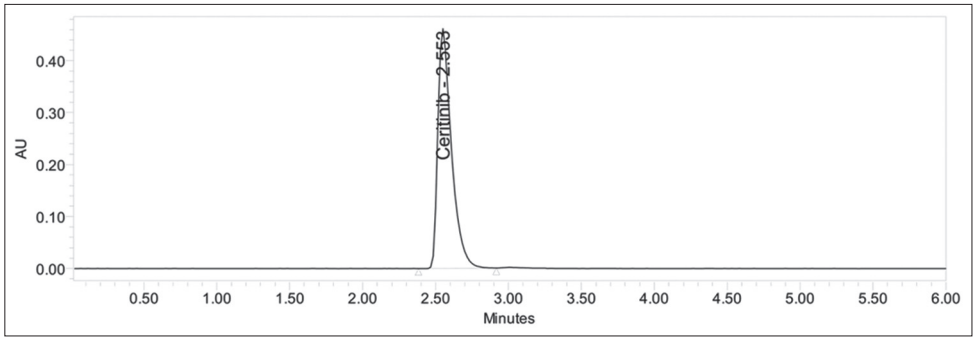

Fig. 12: Method precision chromatogram of ceritinib

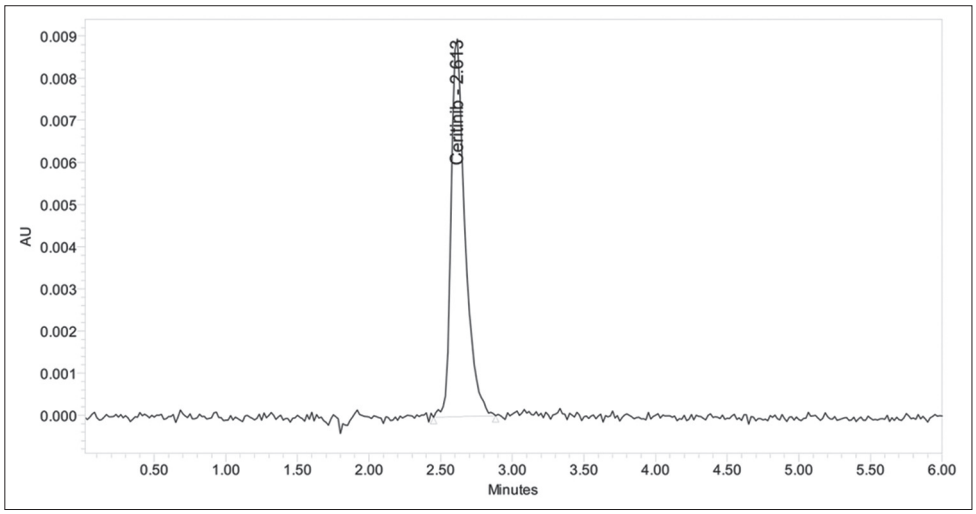

Fig. 13: Chromatogram of limit of detection of ceritinib

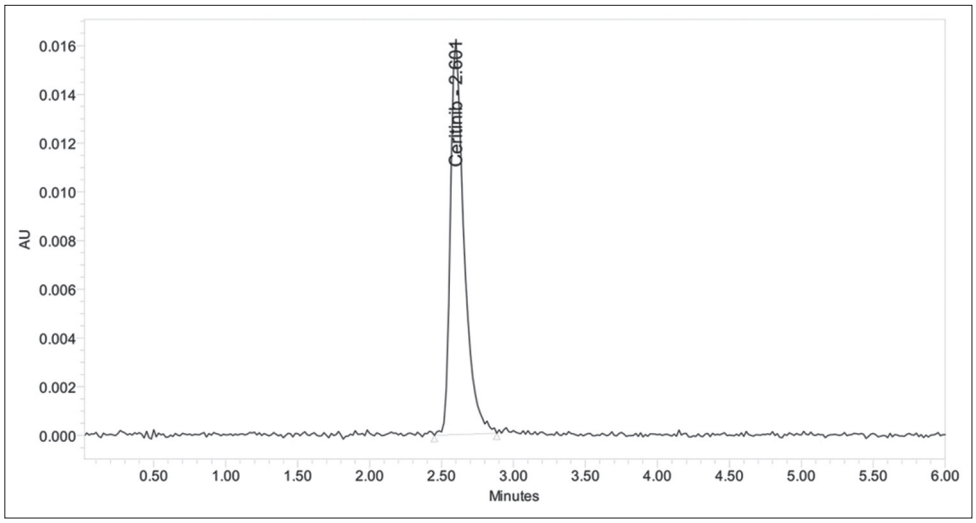

Fig. 14: Chromatogram of limit of quantification of ceritinib

The system suitability was evaluated in each condition of the ceritinib drug and compared the results with method precision results the method was robust for change in flow rate and temperature. No peak was observed at the retention time of ceritinib and the developed method was found to be specific. The sample solution was injected and the amount of ceritinib present in the formulation was calculated from the calibration curve. The optimized chromatographic conditions were mentioned in Table 2.

Method validation

The described method has been validated for linearity, precision, accuracy, specificity, LOD and LOQ, system suitability parameters, ruggedness and robustness, as per the ICH guidelines.

Linearity and range

In the concentration range of 25-150 µg/ml for ceritinib, a standard curve was obtained. A statistical method known as linear regression

Table 4: Accuracy data for ceritinib

	Ceritinib area (μV² seconds)		
	50%	**100%**	**150%**
Injection 1	4506268	6089389	7499293
Injection 2	4511593	6080012	7571129
Injection 3	4502357	6066395	7515139
Average	4506939	6078599	7528520
Amount recovered (μg)	49.3732	101.4713	149.5287
% Recovery	98.75	101.47	99.69

Table 5: Data for method precision of ceritinib

	Ceritinib	Area
	Retention time (minutes)	
Injection 1	2.548	3006324
Injection 2	2.553	3000432
Injection 3	2.553	3058357
Injection 4	2.558	3039681
Injection 5	2.563	3007812
Injection 6	2.565	3005264
Average		3019645
Standard deviation		23636.9
% RSD		0.8

RSD: Relative standard deviation

analysis was used to evaluate the linearity of the curve. To assess the linearity of the proposed method slope, intercept and correlation coefficient (r^2) of the standard curve was calculated and was given in Fig. 9. The results were given in Table 3. From the data obtained for ceritinib, the method was found to be linear within the proposed range. The typical linearity chromatograms were given in Fig. 10.

Accuracy

The closeness of results obtained by the method to the true value for the sample is known as accuracy of the method. The accuracy is expressed in terms of percentage recovery. Recovery % is obtained by the standard addition method. In the present study, recovery studies were carried out at 50%, 100%, and 150% spiked levels. The results of recovery % were given in Table 4 and chromatograms of accuracy were presented in Fig. 11.

Precision

The closeness of replicate results obtained from analysis of the same homogeneous sample is known as precision of the method. The precision of the method was assessed by six replicate injections of 100% test concentrations. The precision was expressed in terms of standard deviation and % RSD. The results were given in Table 5 and a chromatogram of method precision was presented in Fig. 12.

LOD and LOQ

The formulae 3.3 σ/S and 10 σ/S were used to calculate LOD and LOQ, respectively. σ is the mean of the standard deviation of Y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The LOD and LOQ values of ceritinib were found to be 0.02 ppm and 0.06 ppm respectively. The chromatograms of LOD and LOQ of ceritinib were shown in Figs. 13 and 14.

CONCLUSION

The proposed method was completely validated as per ICH guidelines and found to be precise and accurate, as depicted by the statistical data of analysis. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration plots and obedience to Beer's laws. The RSD values, the slopes, and intercepts of the calibration graphs indicate the high reproducibility of the proposed method. The low values of LOD and LOQ indicate that the method can be employed over a wide concentration range for linearity. The stability indicating nature of the proposed method was established by performing forced degradation, which provided degradation behavior of ceritinib under various conditions. Hence, the developed HPLC method is stability indicating and can be used for routine analysis of production samples and also to check the stability of bulk samples of ceritinib.

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