

Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF CERITINIB

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ABSTRACT

The purpose of the present work was to develop new, simple, specific, accurate and precise stability indicating RP-HPLC method for determination of ceritinib. In the present study, stress testing of ceritinib was carried out according to ICH guidelines Q1A (R2). ceritinib was subjected to stress conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Extensive degradation was found to occur in acidic condition. Mild degradation was observed in basic and at thermal conditions. Successful separation of drug from degradation products formed under stress conditions was achieved on a Hypersil BDS C18 column (250×4.6mm, 5.0μ particle size) using acetonitrile: acetate buffer (pH 3.7±0.05) (50:50 v/v), at a flow rate of 1.0mL/min and column was maintained at 40°C. Quantification and linearity were achieved at 272nm over the concentration range of 5-100μg/mL for ceritinib. The Correlation Coefficient was found to be 0.9960. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The developed method will be useful for routine analysis for samples of stability studies in the formulation and development.

Keywords: Stability-indicating, HPLC, ceritinib, validation, stress testing.

INTRODUCTION

Ceritinib, 5-Chloro-N4-[2-[(1-methylethyl)sulfonyl]phenyl]-N2-[5-methyl-2-(1-methyl-ethoxy)-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, ZykadiaTM. Ceritinib is a highly selective inhibitor of an important cancer target, ALK (Heudi, *et. al.*, 2014). Ceritinib, a recently approved drug by Food and Drug Administration, is used for the treatment of late-stage (metastatic) non-small cell lung cancer (Waters, 2014). The recommended dosage of ceritinib is 750 mg administered orally once daily on an empty stomach (Shaw, *et al.*, 2014). The chemical structure of ceritinib (Figure 1).

An ultrafast, sensitive, selective, and robust LDTD-APCI-MS/MS method was

developed for the quantification of ceritinib in human plasma (Lanshoeft, 2015). A stability

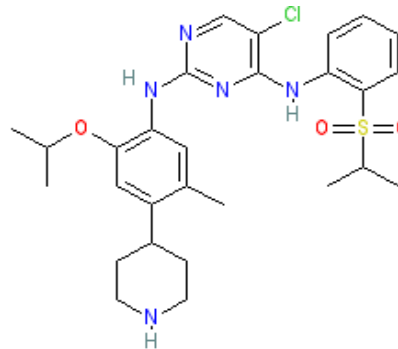


Figure 1. Structure of Ceritinib

indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for estimation of ceritinib was reported (Kumar, 2014). Since, there are only two HPLC method reported in the literature for the estimation of ceritinib in pharmaceutical dosage forms

(Chintala, *et al.*, 2015), (Reddy, *et al.*, 2016), therefore, there is a need to develop quantitative methods under different conditions to achieve improvement in sensitivity, selectivity, as per ICH guidelines (2005). The developed method will be novel for Stability studies and use in the routine analysis for samples in stability study which was very important part of formulation and development.

MATERIAL AND METHODS

Reagents and materials

Ceritinib (purity > 99%) procured from Spectrum lab, Hyderabad, India was used for the study. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and HPLC grade water was used for mobile phase preparation. Nylon membrane filter 0.45 μ syringe filter. Hydrogen peroxide, sodium hydroxide and hydrochloric acid are used of AR grade.

Chromatographic conditions:

The chromatographic separation was achieved on Perkin Elmer (USA) HPLC system (series 200) equipped with Perkin Elmer series 200 pump system having back pressure 5000 psi, manual injector of 20 μ L loop, UV-Visible detector and Hypersil BDS C18 column (250mm x 4.6mm i.d., 5 μ m); Sartorius, analytical balance; An ultra-sonicator; A Shimadzu model 1800 double beam UV/Vis. spectrophotometer with a pair of 10mm matched quartz cells. The mobile phase comprised of acetonitrile: acetate buffer (pH 3.7 \pm 0.05) (60:40% v/v), at a flow rate of 1.0mL/min, and column was maintained at 40°C. The mobile phase was filtered through nylon 0.45 μ m membrane filter and was degassed before being used for analysis. The UV-Visible detector was set at 272nm. The injection volume was 20 μ L and total run time was 10min.

Preparation of the mobile phase

The mobile phase was prepared by mixing 60mL acetonitrile and 40mL acetate buffer (pH 3.7 \pm 0.05) previously filtered through 0.45 μ m nylon membrane filter. The mobile phase was degassed for 15min by sonicating the solution before use.

Preparation of Diluent

Acetonitrile: Water (60:40% v/v) was used as diluent.

Preparation of standard solution

Accurately weighed ceritinib (25mg) was transferred to a 25mL volumetric flask, dissolved in and diluted to the mark with diluent to obtain a standard stock solution (1mg/mL).

Preparation of working standard solution (10 μ g/mL)

Standard solution (0.1mL) was transferred in a 10 mL volumetric flask and diluted up to the mark with mobile phase.

Analysis of tablet dosage form

Twenty tablets (ZYKADIA) were weighed and average weight was calculated. The tablets were finely powdered, and a quantity of powder equivalent to 25mg ceritinib was weighed accurately and transferred to a 25mL volumetric flask containing 15mL diluent, and sonicated for 15min. The solution was allowed to stand at room temperature for 5min and the volume was made up to the mark with diluent to obtain the sample stock solution (1mg/mL). The solution was filtered through 0.45 μ membrane filter. Aliquot (1.0mL) was taken and transferred to 10mL volumetric flask and volume was made up to the mark with diluent to give a solution containing 100 μ g/mL ceritinib. The solution (2.0mL) was transferred to 10 mL volumetric flask and diluted up to the mark with mobile phase to give a solution containing 20 μ g/mL ceritinib. An aliquot (20 μ L) was injected and the chromatogram was recorded. The peak area was noted and the amount of ceritinib was calculated from the regression equation.

Forced degradation study

Ceritinib was subjected to various forced degradation conditions to affect partial degradation of the drug preferably in 20-80% range. The study provided information about the conditions in which the drug was unstable. This Information was useful for development of formulation.

Effect of acid, alkaline and neutral hydrolysis

Accurately weighed ceritinib 25mg was transferred to three different 100mL volumetric flasks and dissolved in diluent (20mL). Hydrochloric acid (0.1N, 10mL), sodium hydroxide (0.1N, 10mL) and water (10mL) were added to separate flasks containing drug samples and mixed properly for acidic, alkaline and neutral degradation respectively and stored at room temperature for 72h.

The samples were neutralized with base or acid as appropriate and diluted up to the marks with diluent to obtain stock solutions (250µg/mL). Dilutions were made with mobile phase to obtain the degraded ceritinib solutions (25µg/mL).

Effect of oxidation

Accurately weighed ceritinib 25 mg was transferred to a 100mL volumetric flask and dissolved in diluent 20mL. Hydrogen peroxide solution (3%) 10mL was added, mixed, and stored at room temperature for 72h. The sample was diluted up to the mark with acetonitrile to obtain stock solution (250µg/mL). Dilution was made with mobile phase to obtain the degraded Ceritinib solution (25µg/mL).

Effect of heat

Ceritinib 25mg was distributed over a glass plate and kept in an oven at 60°C for 72h, then ceritinib was transferred in a 100mL volumetric flask, and dilutions were made with mobile phase to obtain the degraded ceritinib solution (25µg/mL).

Effect of light

Ceritinib solution (prepared by dissolving 25mg ceritinib in 20mL acetonitrile in 100mL volumetric flask) was exposed to sun light for 48h, while ceritinib powder 25mg was exposed to UV light for 48h. After exposure, dilutions were made to obtain the degraded ceritinib solutions (25µg/mL). Aliquots (20µL) of the stressed samples were injected into the HPLC system as described under chromatographic conditions and the chromatograms were recorded.

Method validation

As per the ICH guideline Q2 (R1) (2005), the method validation parameters

namely specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness were studied.

Solution stability

Sample solutions were kept at 25±2°C (24h) and 2-8°C (3 days), respectively. Assay percentage of initial time period was compared with these two time periods. The change in the assay percentage was calculated. The difference between assay results should not be more than 2% for formulation, and 0.5% for active pharmaceutical ingredient (API).

Specificity

Chromatograms of ceritinib solutions and degraded samples were studied in order to provide an indication of the stability indicating properties and specificity of the method. The stress conditions employed were acidic, alkaline, neutral, oxidative, thermal and photolytic degradation. The degraded samples were analyzed against freshly prepared sample solutions.

Linearity (Calibration Curve)

Standard solutions (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3mL equivalent to 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0µg/mL of ceritinib) were transferred in a series of 10mL volumetric flasks and diluted to the mark with mobile phase. An aliquot (20µL) of each solution was injected under the operating chromatographic conditions as described earlier. Calibration curve was constructed by plotting peak areas versus concentrations, and the regression equation was calculated. Each response was average of three determinations.

Accuracy (% recovery)

Accuracy of the method was determined by calculating percentage recovery of ceritinib by the standard addition method. Known amount of standard solutions of ceritinib (0, 5, 10 and 15µg/mL) were added to a pre-analyzed sample solution of ceritinib (10µg/mL). Each solution was injected in triplicate, and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the calibration curve.

Precision

Repeatability was checked by repeatedly ($n = 6$) injecting ceritinib solution ($10 \mu\text{g/mL}$) and recording the chromatogram. Intra-day and inter-day precisions of the developed method was determined by measuring the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of ceritinib (10.0 , 20.0 and $30.0 \mu\text{g/mL}$). The results were reported in terms of relative standard deviation.

Limit of detection and limit of quantification

Limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of response (σ) and slope (S) of the calibration curve.

$$\text{LOD} = 3.3 \times \sigma / S$$

$$\text{LOQ} = 10 \times \sigma / S$$

Robustness

Robustness was studied by analyzing the samples of ceritinib by deliberate variation in the method parameters. The change in the response of ceritinib was noted. Robustness of the method was studied by changing the extraction time of ceritinib from tablet dosage form by ± 2 min, composition of mobile phase by $\pm 2\%$ of organic solvent, wavelength by ± 2 nm, flow rate by ± 0.2 mL/min and column oven temperature by 2°C . The changes in the response of ceritinib were noted and compared with the original one.

System-suitability test

System suitability tests were used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were retention time, tailing factor and theoretical plates of chromatographic peak as RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

Selection of column and mobile phase

As per the published literature and knowledge of the molecule, reverse phase liquid chromatography (RP-HPLC) is suitable for analysis of ceritinib. In case of RP-HPLC various columns are available, but as the main aim of the method was to resolve the

compound from degraded products, C_{18} column ($250 \times 4.6 \text{ mm i.d.}$, $5 \mu\text{m}$ particle size) was preferred over the other columns. Resolution is the most important criteria for the method, it is imperative to achieve good resolution among the compound and degraded products. As per the value of pK_a and solubility of compound various composition of mobile phase were tried.

The chromatographic conditions were optimized with a view to develop a stability indicating assay method, which can separate the drug from its degradation products with good resolution. Mobile phase consisting of acetonitrile: acetate buffer ($\text{pH } 3.7 \pm 0.05$) ($50:50\% \text{ v/v}$) at a flow rate of 1.0 mL/min , was found to be satisfactory to obtain well-resolved peaks with better reproducibility and repeatability for ceritinib (Figure 2).

Method validation

The change in assay results after storage at 25°C (24h) and $2-8^\circ\text{C}$ (3 days) was evaluated. It was found that the difference in assay results was not more than 2% for formulation, and 0.5% for API, indicating stability of ceritinib solution.

The developed analytical method was found to be specific as there was no inference of any related impurities after the stress degradation study (Figure 3). It was shown that the ceritinib peaks were free from excipients and co-eluting impurities.

The linear correlation was obtained between peak area and concentration of ceritinib in the range of $5-30 \mu\text{g/mL}$, the linearity of the calibration curve was validated by the value of correlation coefficient of the regression (r) and the Y intercept were found to be 0.9960 and $Y = 38217x + 72833$ respectively. The regression analysis of the calibration curves (Table I).

The accuracy study was carried out by the standard addition method at level of $\pm 50\%$ of standard concentration. The percent recoveries were found in the range of $98.86-101.12\%$, which indicated accuracy of the method (Table II). The accuracy study had shown results within the limit of $\% \text{ RSD}$ less than 2% .

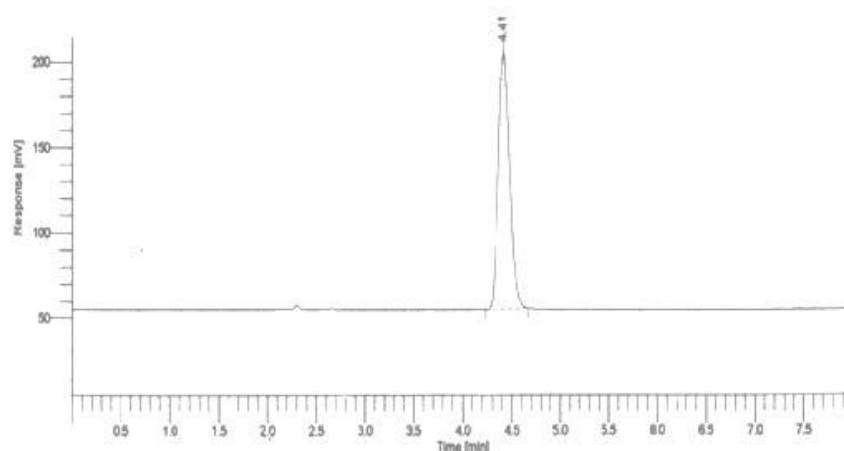


Figure 2. Chromatogram of ceritinib with retention time of 4.41min

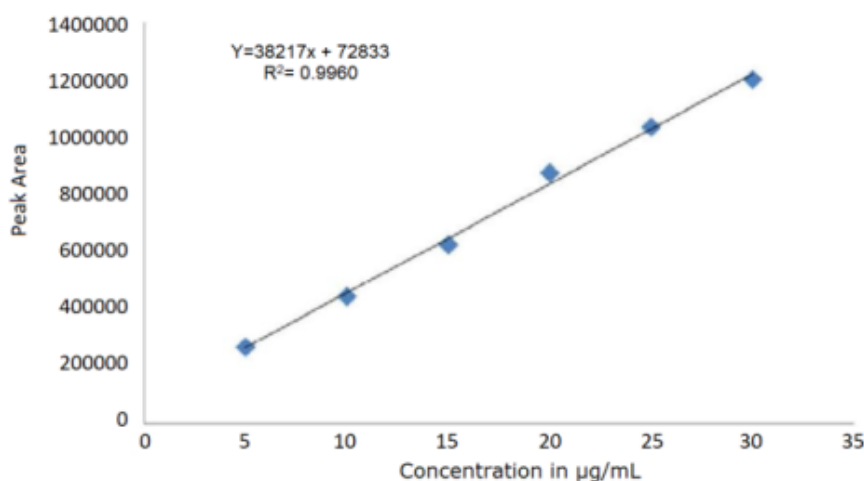


Figure 3. Calibration curve of ceritinib

The % RSD for repeatability (Table III) of ceritinib was found to be 1.25. The value of % RSD for intra-day precision was found to be in the range of 0.93 - 1.15% and inter-day precision was found to be in the range of 1.07 - 1.22 %, which indicated that the method was precise (Table IV). The Precision study showed results within the limit of % RSD less than 2%. The Limit of detection (LOD) for ceritinib was found to be 0.062µg/mL, while the Limit of quantification (LOQ) was 0.187µg/mL. The method was found to be robust as the results were not significantly affected by slight variation in extraction time, composition of mobile phase, wavelength and flow rate of the mobile phase.

System-suitability test

The % RSD of retention time, tailing factor, and theoretical plates were found to be 1.26%, 1.08% and 0.46% respectively. The % RSD of system-suitability test parameters was found satisfactory as % RSD should not be more than 2% (Table V).

Analysis of tablet dosage form

The proposed RP-HPLC method was successfully applied for determination of ceritinib from tablet dosage form. The percentage of ceritinib was found to be 99.07%; which was comparable with the corresponding label claim (Table VI).

Table I. Optical and regression characteristics (n=3)

Parameter	Ceritinib
Linearity range ($\mu\text{g/mL}$)	5-30
Linearity equation	$y = 38217x + 72833$
LOD ($\mu\text{g/mL}$)	0.062
LOQ ($\mu\text{g/mL}$)	0.187
Correlation coefficient (r)	0.9960

Table II. Results of recovery study (n=3)

Amount Taken ($\mu\text{g/mL}$)	Amount added ($\mu\text{g/mL}$)	Amount Found ($\mu\text{g/mL}$)	Recovery \pm S.D, %	% RSD
10	0	10.05	100.50 \pm 0.92	0.92
10	5	14.83	98.86 \pm 1.65	1.65
10	10	19.90	99.50 \pm 1.37	1.37
10	15	25.28	101.12 \pm 0.77	0.77

Table III. Results of repeatability (n=6)

Drug	1	2	3	4	5	6	Mean	SD	% RSD
Ceritinib Peak area	443614.0	454953.1	443275.4	445228.7	448743.8	438564.2	445729.9	5590.19	1.25

Table IV. Results of Intra-day and Inter-day precision (n=3)

Ceritinib ($\mu\text{g/mL}$)	Intra-day precision		Inter-day precision	
	Mean peak area \pm SD	% RSD	Mean peak area \pm SD	% RSD
10	443614.0 \pm 4152.12	0.91	448745.3 \pm 4836.18	1.04
20	875777.0 \pm 9638.35	1.08	878418.6 \pm 9858.21	1.07
30	1200410.0 \pm 13864.16	1.13	1201059.6 \pm 14728.26	1.12

Degradation study

Forced degradation study of ceritinib was carried out under various stress conditions as follows

Effect of acid, alkaline and neutral hydrolysis

Ceritinib was found to undergo 64.67% decomposition under acidic stress condition with a major degradation product at retention time of about 3.88 min and minor degradation product at retention time of about 2.30min and minute decomposition about 3.7% under basic stress condition with a degradation product at retention time of about 2.30min. Under neutral degradation condition, no degradation was observed. Hence, ceritinib was found to be highly degradable in basic condition, and very minute degradable in acidic condition but not

degradable in neutral condition.

Effect of oxidation

In oxidation stress condition, almost 9.5% of ceritinib was degraded and degradation peak appeared in chromatogram at 2.30min retention time.

Effect of heat

Under dry thermal stress condition, ceritinib was degraded about 12.23% with degradation product at retention time of about 2.58 and 6.83min.

Effect of light

When ceritinib in solution state was exposed to sun light; and ceritinib in powder state was exposed to UV light, no degradation was observed, respectively.

Table V. System suitability test parameters (n = 6)

No.	Retention time, Min.	Tailing factor	Theoretical plates
1	4.41	1.49	9282.12
2	4.41	1.49	9254.23
3	4.35	1.48	9237.48
4	4.27	1.49	9187.75
5	4.38	1.47	9265.58
6	4.41	1.45	9176.38
Mean	4.37	1.48	9233.92
SD	0.055	0.016	42.88
% RSD	1.26	1.08	0.46

Table VI. Analysis results of tablet dosage form (n=3)

Formulation	Drug	No. of Injection	Amount Taken	Amount found	Label Claimed	Amount Found per Tablet	% Label Claim
ZYKADIA	Ceritinib	6	30 mg	29.72 mg	15 mg	14.86 mg	99.07

Table VII. Results of stress degradation study

Stress conditions/duration	% Degradation
Acidic/0.1N HCl	64.67
Alkaline/ 0.1N NaOH	3.70
Oxidative/ 3% H ₂ O ₂	9.50
Thermal 60°C	12.23

The samples exposed to acidic, alkaline, neutral, oxidative, thermal and photolytic conditions were colorless. In Photolytic stability, ceritinib was found to be stable showing no degradation. All degradates were resolved from ceritinib peak and the percentage degradation for each condition indicated that there was no interference from degradates in determination of the ceritinib in tablet dosage form. Thus, the proposed, method was found to be "Stability Indicating".

CONCLUSION

An isocratic stability indicating reverse phase liquid chromatographic method has been developed and validated for the estimation of ceritinib in tablet dosage form, the method was found to be specific as there was no interference of any co-eluting impurities after stress degradation study. The proposed method was found to be simple, accurate, precise, sensitive and robust. Hence, it can be used successfully for the routine analysis of ceritinib in pharmaceutical dosage forms, and for

analysis of stability samples obtained during accelerated stability study

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