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# Analytical Method Development and Validation for the Estimation of Ceritinib by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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**A B S T R A C T**

The chromatographic conditions were successfully developed for the separation of Ceritinib by using Kromosil $C_{18}$ 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.182 mins. The % purity of Ceritinib was found to be 99.87%. The system suitability parameters for Ceritinib such as theoretical plates and tailing factor were found to be4146, 1.23, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Ceritinib was found in concentration range of 30µg-150µg and correlation coefficient ($r^2$) was found to be 0.999, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty. LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Ceritinib in API and Pharmaceutical dosage form.

**Keywords:** Ceritinib, HPLC, LOD, LOQ

**A R T I C L E I N F O**

**CONTENTS**



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## 1. Introduction

**Analytical methods:** Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1, 2]. Trial runs are conducted, method is optimized and validated. When alternate method proposed

is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available

**Description of the Various Analytical Methods**

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

**Chromatography:**

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate[7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC.

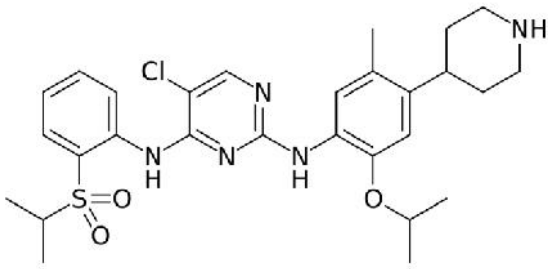


**Figure 1:** Ceritinib

## 2. Experimental

**Apparatus:** The instrument used for the study was Waters

**Apparatus:** The instrument used was WATERS HPLC Auto Sampler, separation module 2695, photo diode array detector 996, Empower-software version-2.

**Reagents & Materials:** The solvents used were Potassium dihydrogen orthophosphate, Methanol, Acetonitril, and Water [10].

**Selection of detection wavelength:**

10 mg of was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained .The overlay spectrum was used for selection of wavelength for ceritinib.

**Selection of mobile phase**

Water: Methanol (35:65) as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved [11].

**Optimization Chromatographic trials for Estimation of Ceritinib by RP- HPLC.**

Optimization Chromatographic conditions

Column : Kromosil C18 4.5×150 mm 5.0 µm
Mobile phase ratio : 65:35% v/v methanol: water
Detection wavelength : 265 nm
Flow rate : 0.8ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 6min
Retention time : 2.162

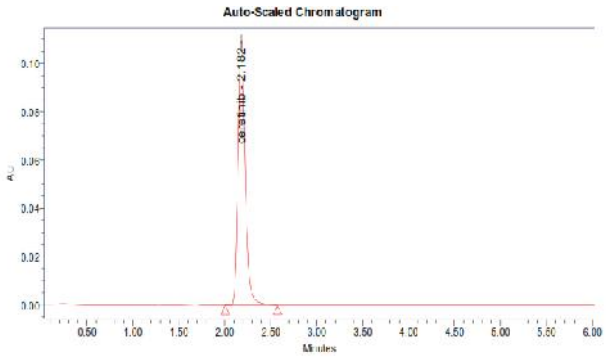


**Figure 2:** Optimization Chromatogram

**Observation:** The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits.

**Procedure**

**Preparation of the individual standard preparation**

10 mg of working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.9 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents [12].

**Preparation of the ceritinib sample solution**

**Sample solution preparation**

10 mg equivalent ceritinib capsule powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 1ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents [13].

**Procedure:** 20µl of the blank, standard and sample were injected into the chromatographic system and areas for the

ceritinib the peak was used for calculating the % assay by using the formulae.

## 3. Results and Discussion

### Method Validation Parameters

**1. Specificity:** The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

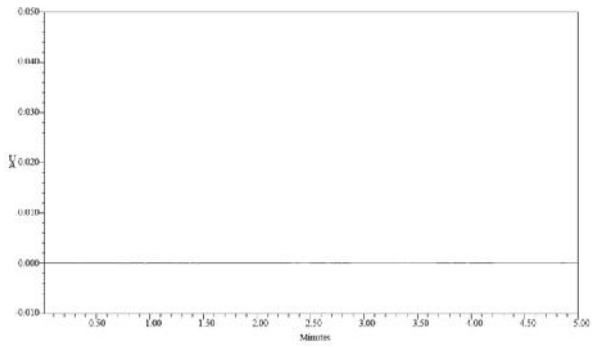

**Figure 3:** Chromatogram of Blank

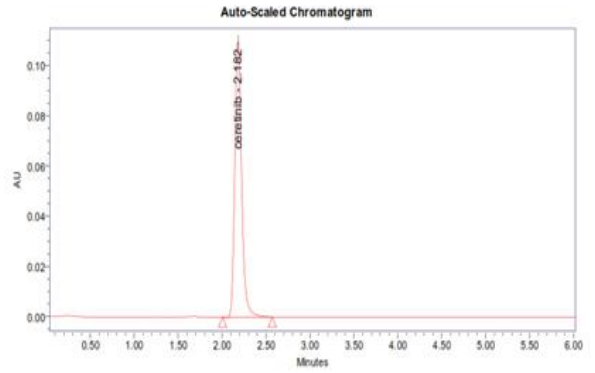


**Figure 4:** Chromatogram of Sample

**2. Linearity:**

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity study was performed for the concentration of 30 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [14].

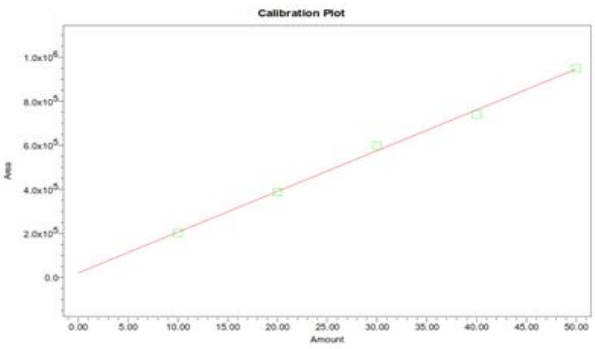


**Figure 4:** Calibration graph of Ezitimibe

**Acceptance criteria:** Correlation coefficient should be not less than 0.999.

**Table 1:** Calibration data of Ceritinib

| Name | Rt | Area |
|---|---|---|
| Ceritinib | 2.178 | 201932 |
| Ceritinib | 2.179 | 338071 |
| Ceritinib | 2.177 | 597859 |
| Ceritinib | 2.186 | 740654 |
| Ceritinib | 2.202 | 950396 |
| Co efficient of correlation ($R^2$) | | 0.997 |

**3. Range**

The linearity study was performed for the concentration of 30ppm to 150ppm. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient

**4. Accuracy**

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there [15].

**Standard addition method**

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

**Percentage method:**

For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively [16].

**Acceptance criteria:**

The mean % recovery of the Ceritinib 100.8 should be not less than 95.0% and not more than 105.0%.

**5. Precision**

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported [17].

**Repeatability**

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Intermediate Precision:**

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Detection Limit**

The LOD was performed for Ceritinib was found to be 2.97 respectively

**Quantitation Limit**

The LOQ was performed for Ceritinib was found to be 9.92 respectively.

**Recovery studies**

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

**Table 2:** Accuracy results for Ceritinib

| %Concentration (at specification level) | Average Area | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 1508773 | 5 | 5.14 | 100.2% | 100.4% |
| 100% | 1866573 | 10 | 10.01 | 98.8% | |
| 150% | 1942321 | 15 | 15.2 | 96.5% | |

**Table 3:** System suitability results for Ceritinib (Flow rate)

| S. No | Flow rate (ml/min) | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.7 | 4352 | 1.1 |
| 2 | 0.8 | 4024 | 1.2 |
| 3 | 0.9 | 3730 | 1.2 |

**Table 4:** Repeatability results for Certinib

| Name | Rt | Area |
|---|---|---|
| Ceritinib | 2.182 | 591196 |
| Ceritinib | 2.177 | 594056 |
| Ceritinib | 2.196 | 594419 |
| Ceritinib | 2.178 | 596875 |
| Ceritinib | 2.191 | 598538 |
| Mean | | 595016 |
| Standard deviation | | 2616.4 |
| %Rsd | | 0.5 |

**Table 5:** Intermediate precision for Ceritinib

| Name | Rt | Area |
|---|---|---|
| Ceritinib | 2.165 | 584681 |
| Ceritinib | 2.181 | 589281 |
| Ceritinib | 2.198 | 596719 |
| Ceritinib | 2.186 | 597658 |
| Ceritinib | 2.188 | 597800 |
| Mean | | 593227 |
| Standard deviation | | 5944.3 |
| %Rsd | | 1.0 |

**Table 6:** The LOD was performed for Ceritinib was found to be 2.97 respectively

| Drug name | Standard deviation (σ) | Slope(s) | LOD(µg) |
|---|---|---|---|
| Ceritinib | 371827.90 | 563365963 | 2.97 |

**Table 7:** The LOQ was performed for Ceritinib was found to be 9.92 respectively.

| Drug name | Standard deviation( σ) | Slope(s) | LOQ(µg) |
|---|---|---|---|
| Ceritinib | 371827.90 | 563365963 | 9.92 |

## 4. Conclusion

A new method was established for estimation of Ceritinib by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ceritinib by using Kromosil $C_{18}$ 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The retention times were found to be 2.182 mins. The % purity of Ceritinib was found to be 99.87%. The system suitability parameters for Ceritinib such as theoretical plates and tailing factor were found to be4146, 1.23, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Ceritinib was found in concentration range of 30µg-150µg and correlation coefficient ($r^2$) was found to be 0.999, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty.LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Ceritinib in API and Pharmaceutical dosage form.

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