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Research Article

Simultaneous Estimation of Ceritinib and Lenvatinib Using RP-HPLC Method

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Abstract

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The assessment of Ceritinib and Lenvatinib was run by RP-HPLC. The Methanol: Phosphate buffer in the ratio of 70: 30% v/v which consists of buffer pH 3.0 was used as a mobile phase. The stationary phase used in the present analysis was Inertsil ODS column (4.6 x 150 mm, 5 µm). The PDA detector with detecting wavelength of 260 nm was used for identification of the separated drugs. The constant flow rate with 1ml/min was employed for efficient separation of the components.

The linearity range of Ceritinib and Lenvatinib was found to be from 100 - 500 ppm of Ceritinib and 1 - 5 ppm of Lenvatinib. Linear regression coefficient was not more than 0.999. The values of% RSD are less than 1% indicating precision of the method. The percentage recovery differs from 98 - 102% of Ceritinib and lenvatinib. It surmised that the method was viewed as simple, linear, precise and accurate as per ICH requirements. The method was viewed as having reasonable application in routine lab analysis with high level of accuracy and precision.

Keywords: Inertsil ODS; Ceritinib and Lenvatinib; RP-HPLC

Introduction

Drugs are crucial for saving and maintaining the quality of human life. For a drug to be used, initially it should be discovered and developed so that it becomes desirable for use by humans. The discovery process of a drug involves its isolation, purification and standardizarion. The development of a drug begins with a solitary compound, which by then advances through various studies intended to help its endorsement as a new drug [1].

Finally the developed drug is formulated into a proper pharmaceutical dosage form. Analysis of the drug is vital as the use of low quality harmful drugs will lead to potential health problems and even loss of human life [2].

Analytical method development and validation is important for every drug to ensure its efficacy, safety and purity [3]. Ceritinb and lenvatinib are drugs belonging to the class of anti-neoplastics kinase inhibitors. Ceritinib is a white crystalline powder which is freely soluble in methanol and in other lower alcohol solvents but practically insoluble in water. Ceritinib acts as an ALK (anaplastic lymphoma kinase) inhibitor. It is used for the treatment of ALK positive metastatic non-small cell lung cancer [4]. Lenvatinib acts as a receptor tyrosine kinase (RTK) inhibitor which inhibits both VEGFR2 and VEGFR3 kinases. It has solubility of 0.122 mg/l in water. It is used in the treatment of thyroid cancer, renal cell carcinoma, hepatocellular carcinoma [5].

Both drugs have potential use in the treatment of cancer. So an attempt was made to develop a cost effective, simple, less time consuming, accurate, precise, linear and efficient RP-HPLC method for simultaneous estimation of ceritinib and lenvatinib in its bulk and pharmaceutical dosage form.

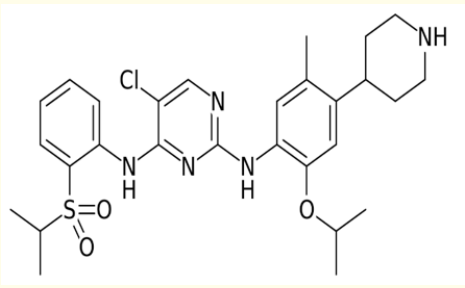

Figure 1: Structure of Ceritinib.

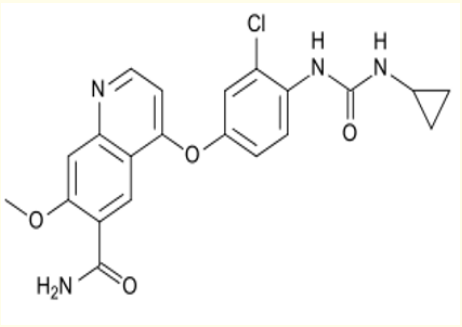

Figure 2: Structure of Lenvatinib.

Literature survey reveals that few analytical methods [6-12] have been reported for the determination of Ceritinib and lenvatinib individually from biological fluids and in pure and pharmaceutical dosage forms. As per the knowledge of the authors, no RP-HPLC method was reported for the simultaneous estimation of the Ceritinib and lenvatinib. Thus, we made an endeavor to develop a simple, accurate, sensitive and precise HPLC technique for the determination of Ceritinib and lenvatinib. The developed strategy has been approved according to the rules of ICH [13].

Methods and Materials

Instrumentation

Waters 2695 HPLC system consisted of a Quaternary pump, Rheodyne injector with PDA detector used for our developed method. Empower 2.0 software, collected, and compiled the chromatographic data obtained for Ceritinib and Lenvatinib in the RP-HPLC systems. Inertsil ODS column (4.6 x 150mm, 5µm) is used for the separation of drugs.

Chemical and reagent

HPLC grade Acetonitrile, Ortho phosphoric Acid, KH_2PO_4, Methanol and HPLC grade distilled water procured from Finer chemical Ltd, Merck, India. Working standards of Ceritinib, Lenvatinib were procured Mylon and Cipla India respectively.

Chromatographic parameters

Equipment: Waters HPLC with separation module 2695

Wavelength: 260 nm

Injection volume: 10 µL.

Flow rate: 1 mL/minute.

Column: particle size)

Mobile Phase: Phosphate buffer of pH 3.0: methanol in the ratio of 30:70% v/v.

Oven Temperature: Ambient

Software: Empower 2.

Preparation of mobile phase

Accurately measure 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) and mix them. Degas in an ultrasonic water bath for 10 minutes and then filter it through 0.45 µ filter under vacuum filtration.

Preparation of standard solution

Precisely weigh and transfer 10 mg of Ceritinib and lenvatinib 10mg of working standard into a 10 mL and 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it totally and make volume sufficient with the same solvent. (Stock solution).

Further pipette 3 ml and 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute sufficient with diluent.

Preparation of sample solution

Accurately weigh 20 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Ceritinib and lenvatinib (marketed formulation) sample into a 10 mL clean dry volumetric flask 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 3 ml of Ceritinib and lenvatinib of the above stock solution into a 10 ml volumetric flask and dilute sufficient with diluent.

Preparation of Phosphate buffer

Accurately weigh 6.8 grams of KH_2PO_4 and take it in a 1000 ml volumetric flask, dissolve and dilute to 1000 ml with HPLC water and adjust the volume to pH 3.0 with Orthophosphoric acid.

Validation

Validation of the method was performed to evaluate the method specificity, linearity, accuracy, precision, percentage purity, limit of detection, limit of quantification and robustness. It was performed as per the guidelines given by ICH.

System suitability

System suitability for the proposed strategy is assessed by injecting the functioning norm of Ceritinib and Lenvatinib into the system. SST parameters like tailing factor, resolution, plate count, retention time were determined to ensure that system is working perfectly fine and it is suitable for analysis in HPLC.

Specificity

Inject blank, standard and sample solutions into the HPLC. To measure the specificity of the system any interferences due to any impurities in analytical peaks are determined.

Linearity

linearity shall be carried out by preparing a standard stock solution and from that solution five levels of preparations of different concentrations are prepared. It shall be performed in the range from 100ppm to 500ppm of Ceritinib and 1ppm to 5ppm of Lenvatinib. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Accuracy (recovery)

Prepare sample solutions of three concentrations i.e, 50%, 100%, 150%. Inject each concentration in triplicate into the HPLC. Calculate the Amount found and Amount added for ceritinib and lenvatinib and calculate the individual recovery and mean recovery values.

Precision

Repeatability

Prepare the standard solution and inject six injections of it for six times into HPLC on the same day and measure the area for all five injections in HPLC. Calculate the% RSD for the area of six replicate injections.

Intermediate precision

To assess the intermediate precision of the method, precision was performed on various day by utilizing different column of same aspects. Prepare the standard solution and inject six injections of it for six times into HPLC and measure the area every one of the six injections in HPLC. Calculate the% RSD for the area of six imitate injections.

Limit of detection

Prepare the lowest sample concentrations of Ceritinib (0.12 µg/ml) and Lenvatinib (0.015 µg/ml) with respect to baseline noise and inject into the HPLC. Calculate the limit of detection for Ceritinib and Lenvatinib based on the signal to noise ratio.

Limit of quantification

Prepare the lowest sample concentrations of Ceritinib (0.42 µg/ml) and Lenvatinib (0.05 µg/ml) with respect to baseline noise and inject into the HPLC. Calculate the limit of detection for Ceritinib and Lenvatinib based on the signal to noise ratio.

Robustness

The flow rate was varied by 0.2 ml/min and Organic composition in the Mobile phase was varied by 10%. The effect of these changes on method was determined.

Results and Discussion

For this study we utilize different mobile phases with different mobile phase composition. The column with different nature includes C18, C8 with different size 25 cm, 15 cm were studied for this developed method. The proposed method Elute the analytes with good percentage of peak area resolution for various analytical parameters at ideal chromatographic conditions like inertial ODS column (4.6 x 150mm, 5µm) with the mobile phase consists of phosphate buffer of pH 3.0: methanol in the ratio of 30:70% v/v. The flow rate was 1ml per minute with a pressure of 2680 psi and detection was at wavelength of 260 nm. The injection volume was

10µl and temperature was ambient. The optimized chromatogram for the standard and sample solution of Ceritinib and Lenvatinib was shown in figure 3 and 4 respectively.

Method validation

Linearity The calibration curve found to be linear (Figure 5 and Figure 6) for Ceritinib and Lenvatinib with concentration range of 100ppm-500ppm for Ceritinib and 1 ppm -5 ppm for Lenvatinib. The correlation coefficient of regression value, concentration and intercept value was calculated using the formula y = 3493x + 10413 (r^2 = 0.999) and y = 37647x + 11553 (r^2 = 0.999) for Ceritinib and Lenvatinib respectively.

Figure 3: Chromatogram of Ceritinib and Lenvatinib standard preparation.

Figure 4: Chromatogram of Ceritinib and Lenvatinib sample preparation.

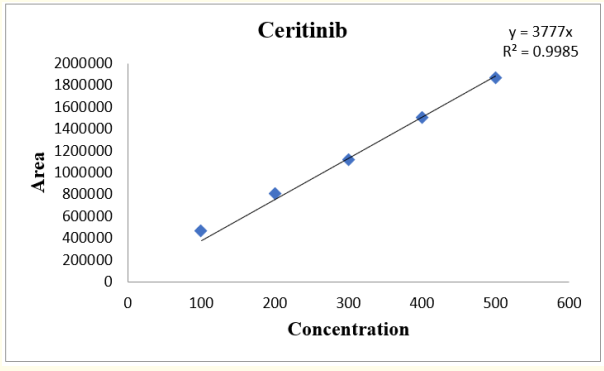

Figure 5

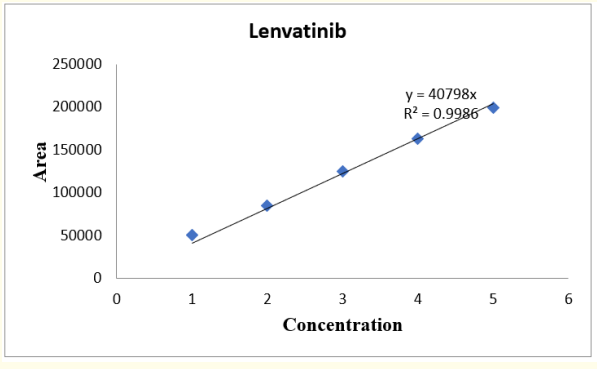

Figure 6: Calibration Curve of Ceritinib and Lenvatinib at 260 nm.

Accuracy

The% Mean recovery for Ceritinib and Lenvatinib was found to be 100.7, 100.8 for 50%, 100, 100.01 for 100% and 98.78, 96.68 for 150% and these results are within acceptable limits. The% RSD for Ceritinib and Lenvatinib were found within limit of ≤ 2 and its high value of recoveries at 50%, 100% and 150% concentrations indicate the performed method is accurate.

Precision

Method precision and Intermediate Precision of Ceritinib and Lenvatinib was calculated by injecting 250 amd 2.5 ppm samples of triplicate solution into HPLC system respectively, the obtained results were found to be more precise. The% RSD of both the methods was found 0.2 for Ceritinib and 0.6 for Lenvatinib indicates that the method was precise and reproducible.

Robustness

The standard and samples of Ceritinib and Lenvatinib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The% RSD was found to be less than 2 for both Ceritinib and Lenvatinib and no significant changes in the entire procedure which indicates the method is Robust.

Limit of detection and limit of quantification

The limit of Detection (LOD) and Limit of quantification (LOQ) for Ceritinib was found to be 2.9 ppm and 10.03 ppm and Lenvatinib was found to be 3 ppm and 10.01 ppm respectively. The very lowest value obtained by this method indicates the developed method was more precise and reproducible.

The validation summary of both Ceritinib and Lenvatinib was given in the table 1.

It surmised that the method was viewed as simple, linear, precise and accurate as per ICH requirements. The method was viewed as having reasonable application in routine lab analysis with high level of accuracy and precision.

S.No.	Validation Parameters	Results for Ceritinib	Results for Lenvatinib
1.	Linearity	100ppm-500ppm	1ppm-5ppm
2.	Correlation coefficient	0.999	0.999
3.	Regression Equation	y= 3493x + 10413	y = 37647x + 11553
4.	Precision (Repeatability)	0.2^	0.6^
5.	Intermediate Precision	0.2^	0.1^
6.	Accuracy	99.84%*	100.51%*
7.	Robustness	Robust	Robust
8.	Assay	99.95%	100.24%
9.	LOQ	10.03	10.1
10.	LOD	2.9	3

Table 1: Summary of HPLC method validation parameters %RSD =^ , MEAN RECOVERY = *.

Conclusion

Our research work on Ceritinib and Lenvatinib tracked down promising in all boundaries in eluting the analyte in exceptionally less time and low organic solvent utilizations.The simple, precise, accurate, cost effective, less time consuming, reliable, reproducible and robust method was validated as per ICH guidelines and we can use it for regular quality control laboratories.

Conflict of Interest

Author declares No conflict of interest for this research work.

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