RESEARCH ARTICLE Am. J. PharmTech Res. 2019; 9(02) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

RP-HPLC Method Development and Validation for Estimation of Lenvatinib In Bulk and Pharmaceutical Dosage Form

Lakshmi Kanth M*[1], Raj Kamal B[2]
1.Research Scholar, Mewar University, Chittorgarh, Rajasthan, India.
2.Research Supervisor, Mewar University, Chittorgarh, Rajasthan, India

ABSTRACT

The objective of present work was to develop and validate a rapid reverse phase high-performance liquid chromatography (RP-HPLC) method for the quantitative analysis of lenvatinib in bulk and pharmaceutical dosage forms. Chromatographic analyses were performed on an ODC column of 250mm 4.6mm: i.d and 5µ particle size with a mobile phase comprising of 0.5M ammonium acetate and acetonitrile in the ratio 90:10 v/v. The flow rate maintained at 1 ml/min, detected lenvatinib at RT 1.15 minutes. The lenvatinib was detected and quantitated using a photodiode array detector at a wavelength of 367 nm. The method was shown to be specific and linear in the range of 20-120µg/ml (r^2= 0.999). The precision (intra- and inter-day) was demonstrated. The method is robust relative to changes in flow rate, column and temperature. The limits of detection and quantitation were 0.4 and 0.12µg/ml respectively. Validation parameters such as specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The method fulfilled the requirements for reliability and feasibility for application to the quantitative analysis of lenvatinib in bulk and pharmaceutical dosage forms.

Keywords: Lenvatinib, thyroid Cancer, ICH guidelines, accuracy, LOD, LOQ

*Corresponding Author Email: kanthmsc06@gmail.com

Received 04 April 2019, Accepted 29 April 2019

Please cite this article as: Kanth LM *et al.*, RP-HPLC Method Development and Validation for Estimation of Lenvatinib In Bulk and Pharmaceutical Dosage Form. American Journal of PharmTech Research 2019.

INTRODUCTION

Lenvatinib is an anticancer drug for the treatment of certain kinds of thyroid cancers. It acts as multiple kinase inhibitors against VEGF-1, VEGF-2, and VEGF-3 kinase. Lenvatinib restrains kinase activities of vascular endothelial growth factor receptors. It also simultaneously restrains other receptors involved in the tumour angiogenesis and proliferation of thyroid cancer including fibroblast growth factor and the platelet derived growth factor receptor alpha [1]. Lenvatinib was first approved in 2015 for the treatment of locally recurrent or metastatic, progressive, radioactive iodine-refractory differentiated thyroid cancer [2]. In May 2016, the US FDA approved it (in combination with everolimus) for the treatment of advanced renal cell carcinoma following one prior anti-angiogenic therapy. Lenvatinib is absorbed quickly from the gut, reaching peak blood plasma concentrations after one to four hours (three to seven hours if taken with food). Bioavailability is estimated to be about 85%. The substance is almost completely (98–99%) bound to plasma protein mainly albumin [3, 4].

Lenvatinib is a multiple receptor tyrosine kinase (RTK) inhibitor indicated for the treatment of thyroid cancer and is available a mesylate salt. Lenvatinib mesylate is chemically known as 4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxyquinoline-6-carboxamide;methanesulfonic acid shown in Figure 1.Its molecular formula is $C_{22}H_{23}ClN_4O_7S$ and molecular weight 522.957 g/mol. (Figure 1).

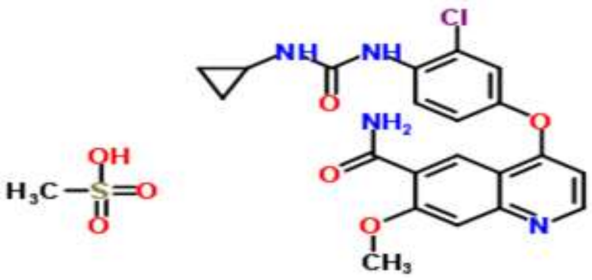

Figure 1: Structure of Lenvatinib mesylate

Literature review reveals that few analytical methods have been reported for the determination of Lenvatinib which includes High performance liquid chromatography and Liquid chromatography-Mass spectroscopy [5-10]. The present study was aimed to develop a novel, simple, economic and validated RP-HPLC method for the estimation of Lenvatinib in bulk and pharmaceutical dosage form according to ICH guidelines [11].

MATERIALS AND METHOD

Materials:

Lenvatinib bulk drug was kindly provided as gift sample by Hetero Drugs, Hyderabad, India. Analytical grade of Ammonium acetate purchased from Rankem Ltd., India and HPLC grade of Methanol purchased from Merck Specialties Private Limited, India. Analytical grade Ortho phosphoric acid purchased from Rankem Ltd., India. Lenvima capsule contain Lenvatinib Mesylate 10mg is obtained from a local pharmacy.

Instrument:

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high-performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with Empower-2 Software. An ODC column of 250mm 4.6mm: i.d and 5µ particle size was used.PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for UV measurements. Semi-micro analytical balance (India), an Ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), Digital pH meter (Systronics model 802) and whatmann filter paper No. 41 (Whatmann International Ltd., England) were used in the study.

Chromatographic Parameters:

Equipment	Waters Alliance e2695 HPLC system with 2998 PDA detector
Column	An ODC column of 250mm 4.6mm: i.d and 5µ particle size
Flow rate	1ml/min
Wavelength	367 nm
Injection volume	20 µl
Run time	7 Minutes

Standard Solution preparation:

Accurately weighed and transferred 10mg of lenvatinib into 10ml dry volumetric flask, 5 ml of diluent added and sonicated for 30 minutes. The solution is made upto the mark using diluent, filtered through 0.45 µm nylon membrane filter to obtain standard stock solution.

Working Standard preparation:

From the above solution 10ml was pipetted out into 100ml volumetric flask and made upto final mark using diluent.

Sample preparation:

Accurately weighed capsule powder equivalent to 10mg of lenvatinib and transferred into 10ml volumetric flask. Diluent added up to the mark, sonicated and filtered through 0.45 µm nylon membrane filter. Further pipette out 10 ml of the above stock solution into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Mobile phase:

The contents of the mobile phase were 0.15g ammonium acetate in 1000ml of water (adjusted the pH value to 7.0 with ammonia solution) and acetonitrile in the ratio of 90:10 v/v. They were filtered before use through a 0.45µm membrane filter and degassed by sonication.

Wave length selection:

Prepared 10 µg/ml concentration of Lenvatinib drug in the mobile phase and scanned by using the UVdouble beam spectrometer with in the wavelength region of 200-400 nm. The maximum absorption observed at 367nm.

Analytical method Validation:

The HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines (2005). The following characteristics were considered for validation: specificity, linearity, range, accuracy, precision, LOD, LOQ and robustness.

The specificity was evaluated by comparing the representative chromatograms of samples containing possible interfering substances and samples containing lenvatinib. Linearity was determined from plot peak area vs. concentration for the five concentrations (20-120 µg/mL). The regression equation and regression coefficient were calculated using least square methodology. The accuracy was tested by calculating the percent recovery of the mean concentration of lenvatinib at three different concentration levels, and the relative standard deviation (RSD) was determined.

Precision is of two types: repeatability or intra-day variability and intermediate precision or inter-day variability. The intraday precession was testing for three different solutions (20, 60,120 µg/ml) on the same day. Inter day precession tested by analyzing solutions of three different concentrations of 20, 60,120 µg/ml six times on different days. The results were reported as %RSD.

The LOD and LOQ were determined from the specific calibration curve obtained using six standard solutions that were the closest to the LOQ.

Robustness was evaluated by deliberately varying the temperature of the analytical column, the flow rate and by using a similar column.

RESULTS AND DISCUSSION:

Linearity:

Linearity was evaluated at five concentration levels ranging from 20-120µg/ml by calculating the regression equation and the correlation coefficient using the method of least squares. The regression equation is $y = 498.79x + 48837.72$ and correlation coefficient is found to be 0.9998 (Table 1).

Table 1: Linearity data for lenvatinib

Concentration µg/ml	Area
20	58965
40	68891
60	78512
80	88541
120	108895

The linearity of the method was evaluated over a concentration range of 20 – 120 µg/ml. The linearity plotted for concentration versus peak area is represented in Figure 2.

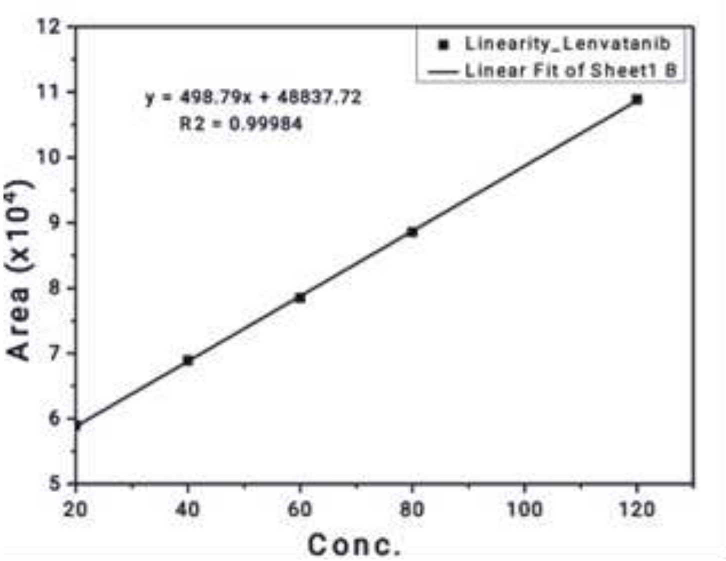

Figure 2: Lenvatinib Linearity Graph

System Suitability:

The system suitability data for Lenvatinib drug evaluated from standard chromatogram shows that the retention time for Lenvatinib drug was 1.15 min. Theoretical plates and tailing factors were found to be 4569 and 1.36 respectively. The obtained results were agreed with ICH guidelines. The results were mentioned in the Table 2. The chromatograms for blank, lenvatinib standard and lenvatinib sample drug are represented in Figure 3, Figure 4 and Figure 5 respectively.

Table 2: System suitability Data for Lenvatinib

Parameter	Value
Retention Time	1.15 min.
Theoretical plates	4569
Tailing factor	1.36

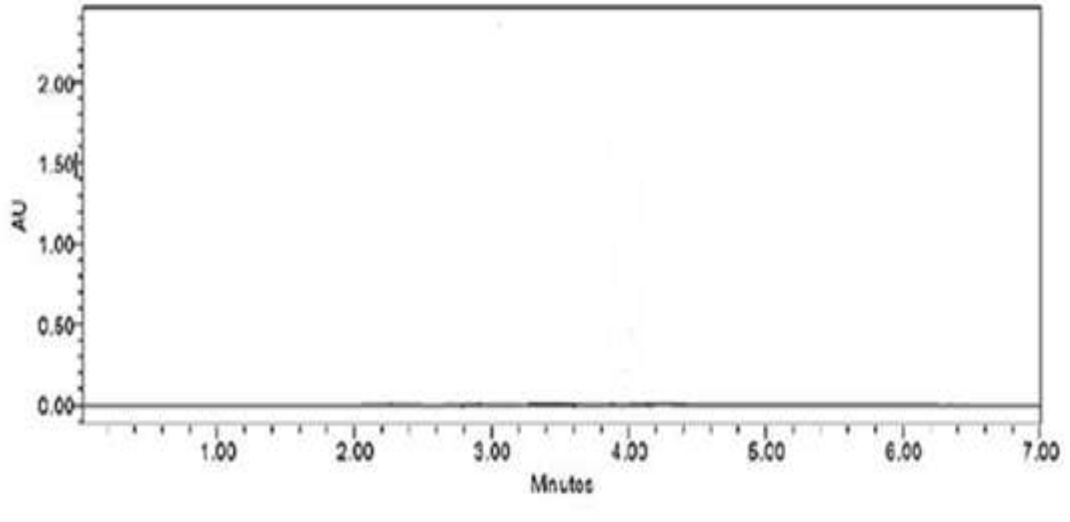

Figure 3: Blank Chromatogram

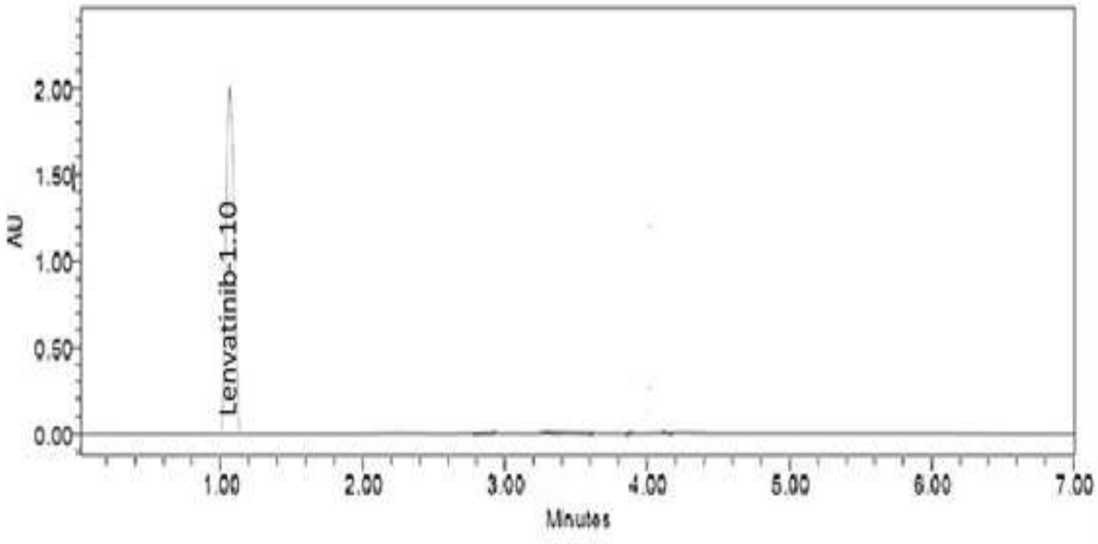

Figure 4: Chromatogram of Lenvatinib Standard

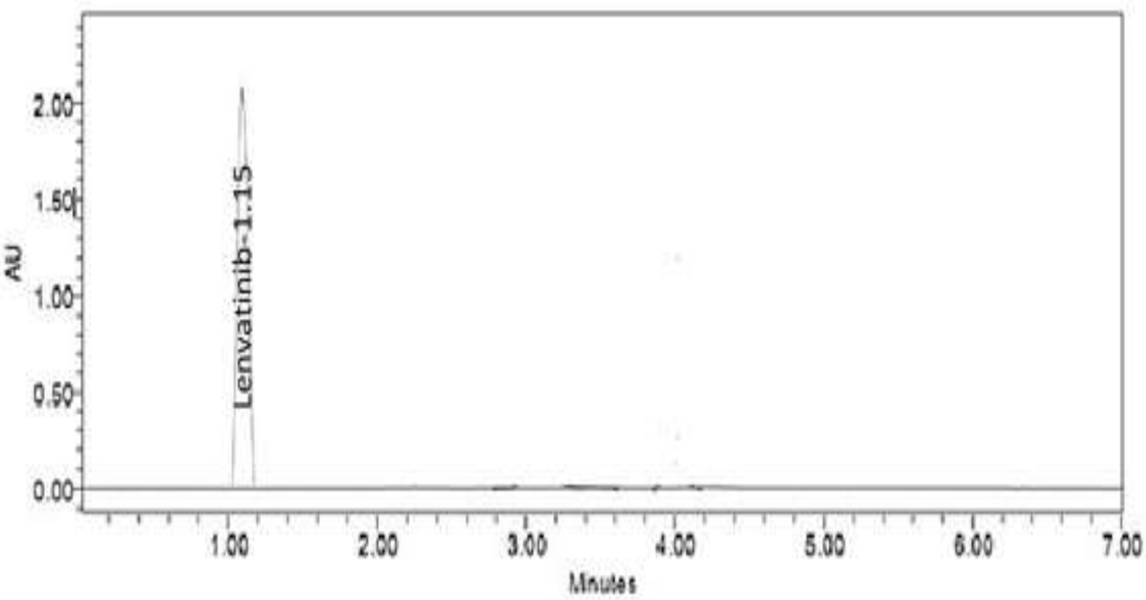

Figure 5: Chromatogram of Lenvatinib Sample

Accuracy:

Accuracy was assessed by calculating the percent recovery and the RSD of the mean concentration of the analyte at three different concentrations. Three standard solutions (20,60, 120µg/ml) were carefully prepared in triplicate and analyzed using the proposed method. Detailed results for these three tested concentration levels are presented in Table 3. The mean percent recovery of lenvatinib from the samples was 99.52% (n = 3).

Table 3: Accuracy data of Lenvatinib

Concentration(µg/ml)	Recovery %	RSD %
20	99.70	0.48
60	99.33	1.31
120	99.55	1.55

Precision:

The precision is a measure of the relative errors of the method, expressed as the RSD for repeatability and intermediate precision. Three concentrations of lenvatinib (20,60, 120 µg/ml) were prepared in triplicate and analyzed on one day or two different days to evaluate intra-day or inter-day variation. The RSDs of responses were calculated in each case and tabulated in Table 4 and Table 5.

Table 4: Intra Day Precision data of Lenvatinib

Concentration (µg/ml)	Measured Conc (µg/ml)±SD	% RSD
20	19.90±0.23	1.19
60	59.57±0.53	0.90

120	120.01±0.21	0.17

Analysis repeatability (n=3)

Table 5: Inter day precision data of Lenvatinib

Concentration (µg/mL)	**Measured Conc(µg/ml)±SD**	**% RSD**
Day 1		
20	19.98±0.11	0.55
60	59.85±0.27	0.45
120	119.71±0.64	0.54
Day 2		
20	20.31±0.29	1.45
60	59.88±0.18	0.30
120	119.93±0.22	0.18

Analysis repeatability (n=3)

mits of detection (LOD) and limit of quantification (LOQ):

The limit of detection was evaluated by serial dilutions of lenvatinib stock solution in order to obtain signal to noise ratio of 3:1 as per ICH guidelines. By using the signal-to-noise method, the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated and signal-to-noise ratio of 3 is used for estimating LOD. This method is commonly applied to analytical methods that exhibit baseline noise. The LOD for lenvatinib were found to be 0.04µg/ml respectively.

$(D_L) = 3.3 \times \sigma / S$ or LOD = Signal/Noise

Units: (µg/ml)

The limit of detection was evaluated by serial dilutions of lenvatinib stock solution in order to obtain signal to noise ratio of 10:1 as per ICH guidelines. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would a signal equal to certain value of noise to signal ratio is estimated and signal to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. The LOQ for lenvatinib were found to be 0.12µg/ml respectively.

$(D_L) = 3.3 \times \sigma / S$ or LOD = Signal/Noise

Units: (µg/ml)

Robustness:

Robustness is a measure of the influence of small changes in the analytical procedures/parameters on the response. The method is robust concerning these alterations in chromatographic parameters.

The maximum RSD obtained was 0.68%. The method is robust concerning these alterations in chromatographic parameters for 20 μg/ml concentration of lenvatinib. (Table 6).

CONCLUSION:

A fast, simple and reliable HPLC method for estimation of lenvatinib using photodiode array diode has been developed and validated according to the ICH guidelines. A satisfactory separation and good peak symmetry for Lenvatinib were obtained with a mobile phase containing a mixture of 0.5M ammonium acetate and acetonitrile in the ratio 90:10 v/v was delivered at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 367 nm based on peak area. The retention time of Lenvatinib was found to be 1.15 minutes. The method fulfilled the requirements to be considered a reliable and feasible method, including specificity, linearity, precision, accuracy, robustness, LOD and LOQ. The analytical procedure has a chromatographic run time of 7 min, which allows analyzing a large number of samples in a short period of time. This method is linear over concentration range of 20-120μg/ml. The accuracy, precession and system suitability values are within the acceptable limit of ±15%.The obtained results suggest that this method can be used for the routine analysis of Lenvatinib drug in the pharmaceuticals.

REFERENCES:

1. Schlumberger M, Tahara M, Wirth L, Robinson B, Brose M, Elisei R et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer .N Engl J Med 2015; 372,621-30.
2. Cserhati T, Szogyi M. Chromatography of anticancer drugs. Eur. Chem. Bull 2013; 2:715-21.
3. Mano Y, Kusano K. A validated LC-MS/MS method of total and unbound lenvatinib quantification in human serum for protein binding studies by equilibrium dialysis. J Pharm Biomed Anal 2015; 114: 82-87.
4. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013; 13: 184–99.
5. Prashanth Y, Mohammed A, Vijaya K, Riyazuddin. Method development and validation of lenvatinib drug by RP-HPLC in pharmaceutical drug dosage form. IAJPS 2016; 3 (10): 1078-85.
6. Sreenivasa C, Sridhar C, Chandra S, Kothapalli Banoth. Analytical methods for the recently approved FDA new molecular entities – a Review .J. Compr. Phar 2016; 3(3):70-82.

7. Srikanth I, Prameela A. Development and validation of liquid chromatography coupled with tandem mass spectrometry method for estimation of lenvatinib in human plasma. Asian J Pharm Clin Res 2017; 10(7): 120-26.
8. Tomoko O, Morita, YoshiyukiSano, TomokaOkano, HirofumiFujii, MakotoTahara, et al.Validation of a Liquid Chromatography-Tandem Mass Spectrometric Assay for Quantitative Analysis of Lenvatinib in Human Plasma. Int J Anal Chem 2017: 2341876.
9. Uttam Prasad P,Sunil Kumar A. A novel validated RP-HPLC-DAD method for the estimation of Lenvatinib Mesylate in bulk and pharmaceutical dosage form. J Chem Pharm Res 2015; 7(9):872-81.
10. Sunil J, Ramreddy G, Srinivas M, Sudhana N . Method Development and Validation for Simultaneous Estimation of Dasatinib and Lenvatinib by Using RP-HPLC in Pharmaceutical Dosage Form. Inventi Rapid: Pharm Analysis & Quality Assurance 2017;4: 1-5.
11. International Conference on Harmonization, ICH Guidelines, Validation of Analytical Procedures Technical Requirements for Registration of Pharmaceuticals for Human Use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.