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Development and Validation of Reverse Phase High Performance Liquid Chromatographic Method for Quantitative Estimation of Lenvatinib in Capsule Dosage Form

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ABSTRACT

The aim of the work is to develop a simple, accurate, precise, and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the determination of anticancer drug Lenvatinib in capsule dosage form. Chromatography was carried out on an Inert Sustain C18 (250 mm*4.6 mm*5 µm) column with a mobile phase consisting of Water : Acetonitrile : Trifluoroacetic acid (60:40:0.1) v/v. The detection was carried out at 241 nm, 10 µl injection volume was selected with a flow rate of 0.8 ml/min. Retention time was found to be 4.15 minutes. Method was linear over the range of 10-100 µg/ml with regression coefficient 0.9991. The method was validated as per ICH guidelines. The developed method is superior in terms of theoretical plates and it has got less tailing factor. The method can be applied for routine quality control analysis of Lenvatinib in capsule formulation.

INTRODUCTION

Lenvatinib is an anti-cancer drug that is used for the treatment of certain kinds of thyroid cancer, and possibly for other cancers as well. It acts as a multiple kinase inhibitor against the vascular endothelial growth factor receptor 1 (VEGFR1), vascular endothelial growth factor receptor 2 (VEGFR2) and VEGFR3 kinases [1]. The chemical name of Lenvatinib is 4-[3-chloro-4-(cyclopropylcarbamoylamino) phenoxy]-7-methoxyquinoline-6-carboxamide Figure 1 and its molecular formula is C21H19ClN4O4. The molar mass of Lenvatinib is 426.857 g/mol. Lenvatinib capsules (Lenvima, Eisai, Inc.), in combination with everolimus, was approved by the U.S. Food and Drug Administration on May 13, 2016, for the treatment of advanced renal cell carcinoma following prior anti-angiogenic therapy. Lenvatinib was first approved in 2015 for the treatment of locally recurrent or metastatic, progressive, radioactive iodine refractory differentiated thyroid cancer [2,3] .

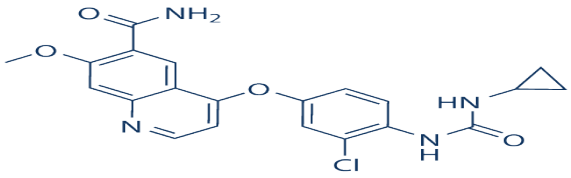

Figure 1: Structure of Lenvatinib.

Literature survey reveals that very few chromatographic methods were developed for the estimation of Lenvatinib using buffer system with very low retention times and low system suitability parameters [4,5]. Hence, attempts were made to develop a simple, rapid, precise and accurate reverse phase chromatographic method to estimate Lenvatinib in capsule dosage form. The proposed method was optimized and validated according to International Conference on Harmonization (ICH) guidelines [6].

The objective is to give an overview of the mechanism of Reverse Phase High Performance Liquid Chromatography (RP-HPLC) of Lenvatinib and explain the basis of the retention mechanism and to achieve high speed separation without any loss of reproducibility.

MATERIALS AND METHODS

Chemicals and reagents: An analytically pure Lenvatinib standard was procured from Central Drugs Testing Laboratory, Mumbai with defined potency [99.8 (as is basis)]. LENVIMA (10 mg) capsules were received as gift sample from Assistant Drugs Controller office, Air Cargo, Mumbai. Acetonitrile of HPLC grade from Merck and Trifluoroacetic acid of analytical grade from Sisco Research Laboratories Pvt. Ltd were used for the preparation of mobile phase and other solutions. Milli-Q water was used to prepare all the required solutions [4].

Instrumentation: Perkin Elmer UV/VIS Spectrometer Lambda 25 connected to a computer loaded with software Perkin Elmer UV Win Lab was used for all the spectrophotometric measurements. The chromatographic method was performed on Perkin Elmer Flexar HPLC using software TC Nav/ver 6.3.2 with LC instrument control. An Inert sustain C18 (250 mm×4.6 mm×5µm) column was used as a stationary phase.

Diluent Preparation: Mixture of water and acetonitrile in the ratio of 1:1 v/v was used for the preparation of standard and sample solutions.

Determination of wavelength: The standard solution (50 µg/ml) of Lenvatinib was scanned in the range of 200-400 nm

against diluent as a blank. Lenvatinib showed maximum absorbance at 241 nm. So the suitable wavelength selected for the HPLC analysis of Lenvatinib was 241 nm Figure 2.

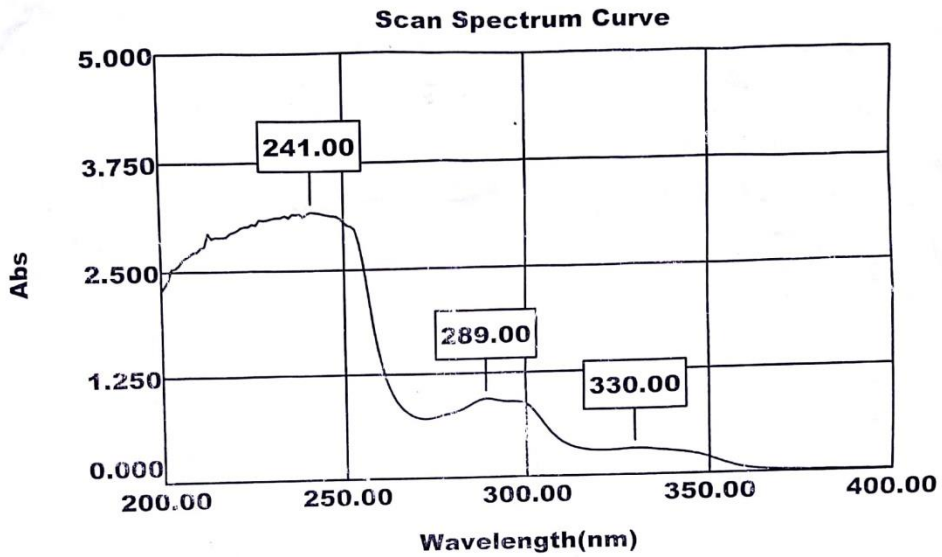

Figure 2: UV Spectra of Lenvatinib

Mobile phase preparation: The mobile phase was prepared by mixing Water : Acetonitrile : Trifluoroacetic acid in the ratio of (60:40:0.1) v/v. It was then sonicated using ultrasonic bath for 10 minutes and was filtered using 0.2 µm nylon filter.

Preparation of Standard solution: 10 mg of standard Lenvatinib was accurately weighed and dilutions were made in the diluent to get a concentration of 50 µg/ml, which was treated as 100% target concentration [5].

Preparation of Sample solution: Sample (LENVIMA 10 mg) equivalent to 10 mg of Lenvatinib was accurately weighed and transferred into a 100 ml volumetric flask. Approximately 50 ml diluent was added and the solution was sonicated for 20 minutes. The flask was then filled upto the mark with diluent, mixed and filtered. After filtration, 10 ml was pipetted out from the solution and was taken in a 20 ml volumetric flask. It was then diluted upto the mark with diluent to get a concentration of 50 µg/ml of Lenvatinib.

RESULTS

Method optimization: Lenvatinib is a basic and a non-polar drug. A base deactivated column Inert sustain was selected for retention of this drug. Here different columns and different compositions of mobile phase solvents were used on trial and error basis. Initial trials were made with mobile phase consisting of water and acetonitrile with different ratios but high retention times with poor peak shapes were observed. Good peaks were obtained with mobile phase consisting of Water : **Acetonitrile :** Trifluoroacetic acid in the ratio of (60:40:0.1) v/v. The flow rate was kept as 0.8 ml/min and UV detection was carried out at a wavelength of 241 nm. All determinations were performed at a constant column temperature (35^0 C). 10 µl of the standard preparation and sample preparation were separately injected and chromatographed.

Method validation

The developed method was validated as per the ICH guidelines for the parameters like specificity, linearity, precision, accuracy, robustness.

System suitability: System suitability tests are an important part of method development. Before starting sample analysis the chromatographic systems used for analysis must pass the system suitability limits. A blank preparation (single injection) and standard preparation (six replicates) at the working concentration (50 µg/ml) were injected into the HPLC and the

chromatograms were recorded to evaluate the system suitability parameters like area, Retention Time (RT), number of theoretical plates (N) and tailing factor. The results are given in Table 1.

TABLE 1: System suitability studies of Lenvatinib.

Sr. No.	Area	Retention Time	Theoretical Plates	Tailing Factor
1	3647364	4.145	8170	1.243
2	3639670	4.148	8163	1.247
3	3643183	4.147	8176	1.248
4	3645110	4.147	8149	1.250
5	3642978	4.149	8099	1.252
6	3646228	4.151	8100	1.254
AVERAGE	3644089	4.15	8143	1.25
SD	2754.128	0.0020		0.0039
%RSD	0.08	0.05		0.31
Limit	NMT 2.0 %	NMT 1.0 %	NLT 2000	NMT 2.0 %

The optimized chromatographic condition was found satisfactory to yield well retained, sharp and symmetrical peak at 4.15 minutes. The average number of theoretical plates were 8143 and tailing factor was 1.25 for Lenvatinib, which indicates efficient performance of the column.

Specificity: For Specificity blank, placebo, standard drug solution (50 µg/ml) and sample solution (50 µg/ml) were injected into the HPLC and their chromatograms were recorded. Figures 3- 6 represents the chromatograms of blank, placebo, standard drug solution and sample solution respectively.

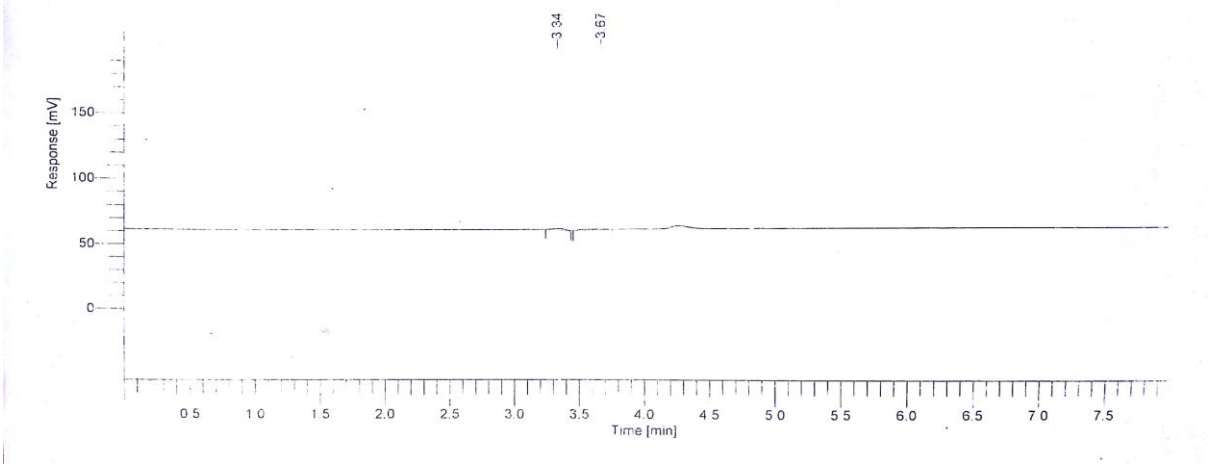

Figure 3: Chromatogram of Blank solution.

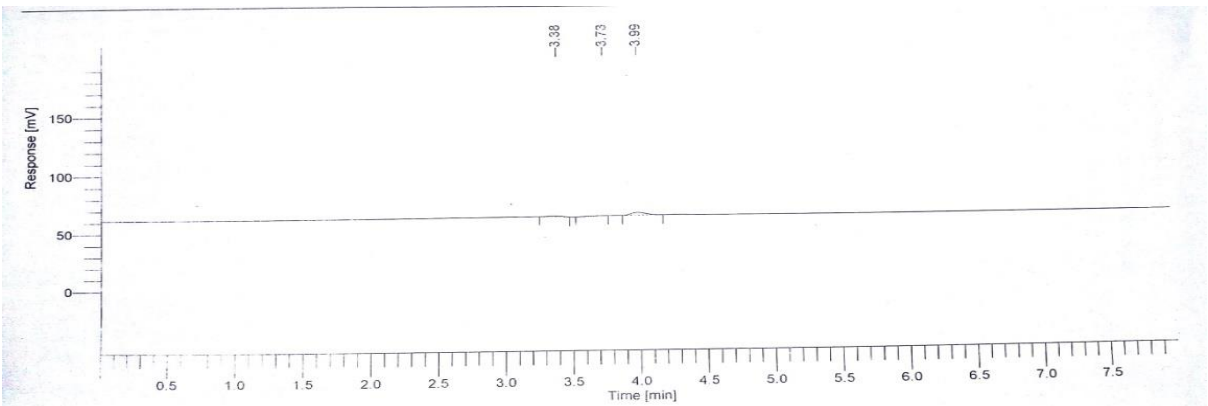

Figure 4: Chromatogram of Placebo solution.

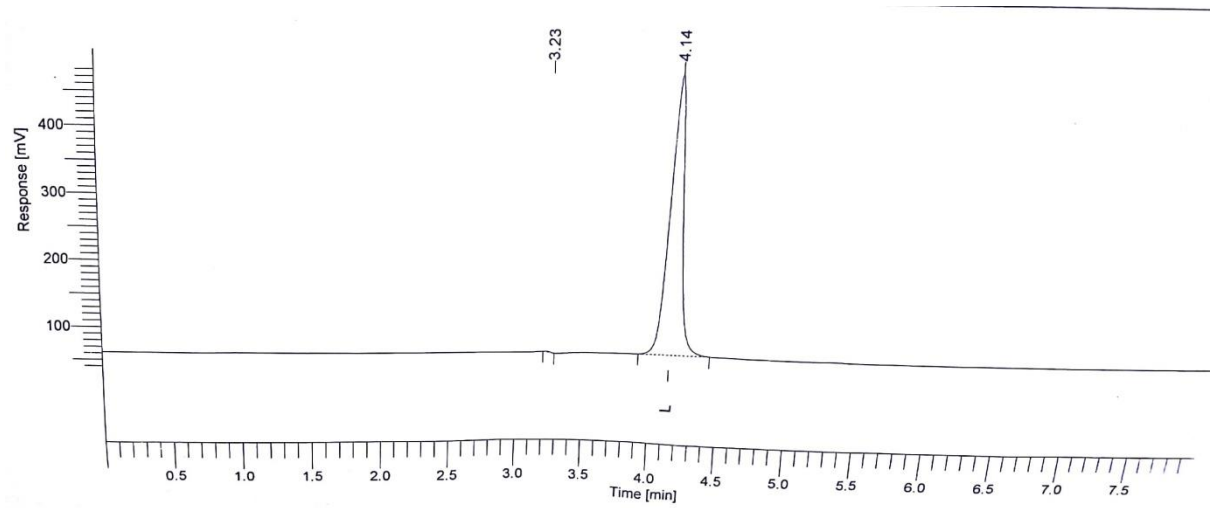

Figure. 5: Chromatogram of Lenvatinib standard solution.

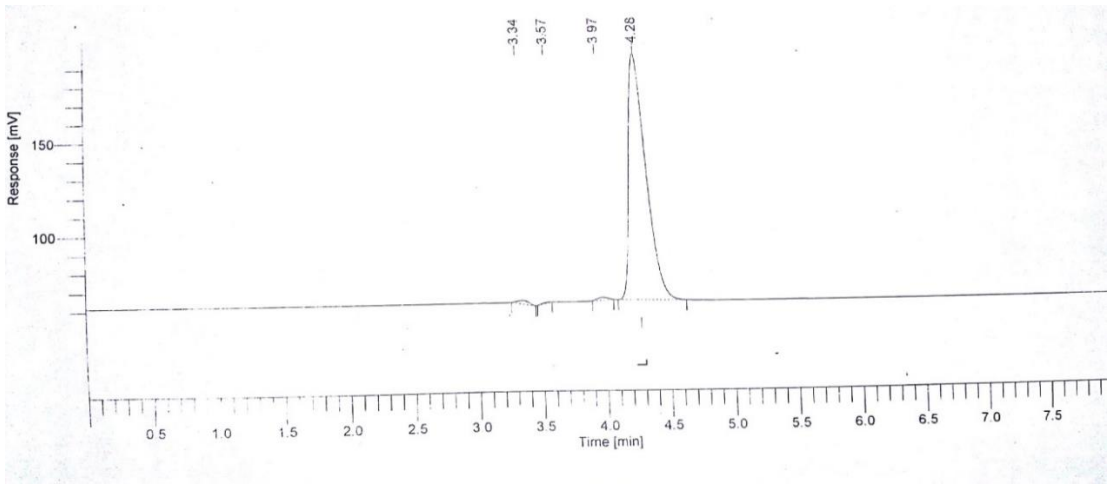

Figure. 6: Chromatogram of Lenvatinib sample solution.

The chromatograms of blank, placebo, standard drug solution and sample solution indicate that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drug as blank and placebo have no peak at the retention time of Lenvatinib. From the above results, the method developed is found to be specific.

Linearity: The linearity of the method was calculated by injecting the standard solution of Lenvatinib of the concentrations of 10, 20, 30, 50, 70, 80, 100 µg/ml prepared from the standard stock solution (1000 µg/ml). A linearity plot was plotted by taking concentration level of drug on X-axis and the corresponding peak area on Y-axis. Results are shown in (Table 2). The correlation coefficient for Lenvatinib was found to be 0.9991 that is not less than 0.999. Hence, the response is said to be linear from 10-100 µg/ml.

Precision

System Precision: Six injections of the standard solution (50 µg/ml) were injected into the HPLC. The average, standard deviation and relative standard deviation (RSD %) of the peak areas of six replicate injections were calculated and reported. The summarized results are shown in Table 3.

TABLE 3: System precision data of lenvatinib

Injection no.	Area
1	3543108
2	3657934
3	3673545
4	3667287
5	3674373
6	3647364
AVERAGE	3643935
SD	50442.75
%RSD	1.3843
Limit	NMT 2.0 %

Method precision: Six replicate injections of standard solution (50 µg/ml) and six sample preparations of Lenvatinib (50 µg/ml) were prepared and injected in duplicate into the chromatographic system. The mean assay percentage of Lenvatinib sample solution was calculated and reported along with standard deviation and relative standard deviation of the six samples. The results are shown in Table 4.

TABLE 4: Method precision data of Lenvatinib.

Injection. No	% Assay
1	99.4
2	98.4
3	99.5
4	99.2
5	99.0
6	100.4
AVERAGE	99.3
SD	0.6585
%RSD	0.6631
Limit	NMT 2.0 %

Intermediate precision: Six replicate injections of standard solution (50 µg/ml) and six sample preparations of Lenvatinib (50 µg/ml) were prepared and injected in duplicate on two different days, by two different analysts and in two different HPLC systems. The assay results are shown in Table 5.

TABLE 5: Intermediate precision data of Lenvatinib.

Injection. No	Analyst A (Day 1)HPLC 1	Analyst B (Day 2)HPLC 2
	% Assay	% Assay
1	99.4	100.3
2	98.4	99.3
3	99.5	99.5
4	99.2	100.2
5	99.0	100.1
6	100.4	101.0
AVERAGE	99.3	100.1
SD	0.6585	0.6088
%RSD	0.6631	0.6084
Limit	NMT 2.0 %	NMT 2.0 %

According to the results of system precision, method precision and intermediate precision the % RSD values are not more than 2%. So, it can be concluded that the method is precise.

Accuracy: Accuracy was determined by the method of standard addition method, by the determination of % mean recovery of sample at three different levels (110, 120 and 130%). At each level, three determinations were performed. The percent mean recovery was found out as per the calculations shown in Table 6.

TABLE 6: Accuracy studies of Lenvatinib.

Level	Spiked Level (mg)	Amount Recovered (mg)	% Recovery	Mean	SD	%RSD
110%	11.3	11.15	98.67			
110%	11.3	11.27	99.73	99.17	0.5334	0.5379
110%	11.3	11.20	99.12			
120%	12.2	12.08	99.02			
120%	12.2	12.18	99.84	99.95	0.9881	0.9887
120%	12.2	12.32	100.98			
130%	13.4	13.31	99.33			
130%	13.4	13.51	100.82	99.78	0.9079	0.9099
130%	13.4	13.29	99.18			

For accuracy studies the limit for percent mean recovery is 98% - 102%. From the results, it can be seen that the percent mean recovery is within the limit, hence the method is accurate.

Robustness: The sample of Lenvatinib was analyzed using the developed method after a deliberate change in detection wavelength, flow rate and column temperature. Three sample preparations of 50 µg/ml were prepared and injected in duplicate along with six replicate injections of standard solution of 50 µg/ml under different chromatographic conditions. The method was sufficiently robust for normally expected variations in chromatographic conditions such as wavelength, temperature and flow rate as shown in Table 7.

TABLE 7: Robustness data of Lenvatinib.

Parameter	Change in parameter (±)	% Estimation	Mean	SD	%RSD
Wavelength	239	99.4			
(± 2 nm)	241	99.3	99.7	0.6658	0.6676
	243	100.5			
Flow rate	0.6	100.6			
(± 0.2 ml)	0.8	99.3	100.3	0.8505	0.8482
	1	100.9			
Temperature	33	100.9			
(± 2 ˚C)	35	99.8	100.0	0.7767	0.7765
	37	99.4			

Assay

The optimized method was applied to the assay of Lenvatinib sample. Five replicate injections of standard preparation (50 µg/ml) and six sample preparations (50 µg/ml) (each in duplicate) were injected into the chromatographic system and the responses for Lenvatinib peaks were measured.

The drug content was calculated as an average of six determinations and assay results are shown in Table 8. The optimized method was applied to the assay of Lenvatinib sample.

TABLE 8: Assay results of lenvatinib sample.

Sr. No.	Weight of standard (mg)	Sample Weight (equivalent to 10 mg)	*Area of standard at 241 nm	Area of sample at 241 nm	% Assay
1	10.1	101.2	3644089	3597754	99.4
2		102.5		3606958	98.4
3		101.4		3606848	99.5
4		101.6		3605604	99.2
5		102.7		3636997	99.0
6		101.1		3631001	100.4
Mean					99.3
± SD					0.65
% RSD					0.65

The result obtained showed that the percentage recoveries were high and SD values were low, which confirms the method is suitable for routine determination of Lenvatinib in its pharmaceutical preparation.

DISCUSSION

The developed HPLC method is simple, specific, accurate and precise for estimation of Lenvatinib in capsule dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity and recovery in accordance with ICH guidelines.

CONCLUSION

Thus the described method is suitable for routine analysis of Lenvatinib in capsule dosage forms as the percentage recoveries were high and SD values were low, which confirms the method is suitable for routine determination of Lenvatinib in its pharmaceutical preparation.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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