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Original article

RP-UFLC Method Development and Validation for Quantification of Lenvatinib Mesylate in Bulk and Pharmaceutical Formulation

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ABSTRACT:
A rapid and accurate isocratic reverse-phase ultra-fast liquid chromatographic method has been developed for the estimation of Lenvatinib mesylate in bulk and pharmaceutical dosage form. Chromatographic analysis was performed using Target sil C-18 column of 4.6mm 250mm: i.d and 5µ particle size with methanol as a mobile phase at the flow rate of 1mL/min. The column was maintained at ambient temperature (27°C). The Lenvatinib was detected and quantified using a diode array detector at a wavelength of 243nm. In the run time of 5mins Lenvatinib was detected at RT 2.761mins.The method was specific and linear in the range of 1-6µg/mL (r2=0.999), and the limit of detection and limit of quantitation were found to be 6.17 and 18.7µg/mL respectively. Validation parameters such as specificity, linearity, precision, accuracy, robustness, LOD and LOQ were evaluated for the method according to the International Conference for Harmonization (ICH) Q2 R1 guidelines. The method fulfilled requirements for reliability and feasibility for application to the quantitative analysis of Lenvatinib in bulk and pharmaceutical dosage forms.

Keywords: Lenvatinib, thyroid Cancer, RP-HPLC, Methanol, Validation.

1. INTRODUCTION

Lenvatinib is an anticancer drug, acts as a multiple kinase inhibitor against VEGF-1, VEGF-2, and VEGF-3 kinase. Lenvatinib restrains the kinase activities of vascular endothelial growth factor receptors [1, 2].Its primary mechanism of action involves inhibiting signal pathways such as vascular endothelial growth factor receptors (VEGFR) and fibroblast growth factor receptors (FGFR), thereby reducing tumor cell proliferation and angiogenesis and affecting the tumor's immune microenvironment [3]. Lenvatinib was first approved in 2015 for the treatment of locally recurrent or metastatic, progressive, radioactive iodine-refractory differentiated thyroid cancer [4]. 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 [5, 6]. 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 [7]. Lenvatinib Mesylate is chemically known as 4-[3-chloro-4-(cyclopropylcarbamoylamino) phenoxy] 7 methoxy quinolone-6-carboxamide: methane sulfonic acid. Its molecular formula is C22H23ClN4O7Sand molecular weight is 522.957 g/mol (Fig 1).

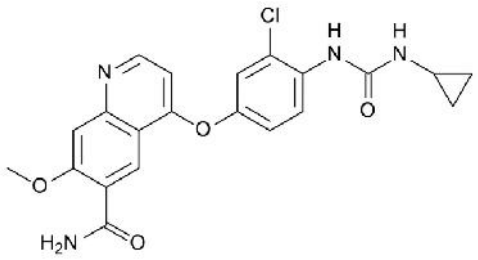

Fig 1: Structure of Lenvatinib mesylate

The literature review reveals that few analytical methods have been reported for determining Lenvatinib including UV, HPLC, UFLC, LC-MS [8]. The present study aimed to develop a novel, simple, economical and validated the developed RP-UFLC method for the estimation of Lenvatinib in bulk and pharmaceutical dosage form according to ICH guidelines.

2. MATERIALS AND METHODS

Shimadzu UV 1800s was used to detect the λmax of Lenvatinib. Shimadzu LC-20AD model with PDA detector

was used for the quantitative analysis of Lenvatinib in bulk and formulation.

Chemicals used:

Lenvatinib Mesylate and capsules (4mg) were procured from Natco pvt. labs, Hyderabad. HPLC grade solvents like Methanol, Ethanol, Acetonitrile and water were used.

Preparation of stock solution:

About 1mg of Lenvatinib Mesylate was weighed and transferred into a 10mL volumetric flask and the sample is dissolved with 10mL of methanol (100µg/mL.) [9].

Preparation of standard solution:

From the stock solution 1mL was pipetted out into the 10mL volumetric flask and made up to the final mark using diluent (10µg/mL).From the above solution 1mL was pipetted out into a 10mL volumetric flask and made up to the mark using a diluent (1µg/mL).

Preparation of sample solution:

62.5 mg of Lenvatinib Mesylate was dissolved in 100 mL methanol. From this 0.1mL was pipetted out into 10ml volumetric flask and made up to the mark with methanol in a 10mL.

Procedure:

Inject 25 µL of the standard, sample into the chromatograph and measure the area for the Lenvatinib Mesylate peak and calculate the %Assay by using the formulae.

3. RESULTS AND DISCUSSION

Selection of Solvent and Wavelength:

Lenvatinib mesylate was evaluated for solubility in various solvents, including distilled water, ethanol, methanol, and acetonitrile [10]. Among these, methanol demonstrated the best solubility and was therefore selected as the solvent for further analysis. For wavelength selection, initial stock and standard solutions were prepared using acetonitrile; however, the resulting spectra showed fluctuations and disturbances. Subsequently, methanol was used to prepare the samples, which yielded clear and stable spectra with a maximum absorbance (λmax) at 243 nm. Hence, 243 nm was chosen as the detection wavelength for the analysis [11].

Optimised Chromatographic Conditions:

The chromatographic analysis was carried out using a reversed-phase C18 column (4.6 × 250 mm, 5 µm) with 100% methanol as the mobile phase. The flow rate was maintained at 1.0 mL/min, and the injection volume was 25 µL. The total run time was set to 5 minutes. Detection was performed at a wavelength of 243 nm, and the compound of interest exhibited a retention time of 2.761 minutes [12] (Fig 2).

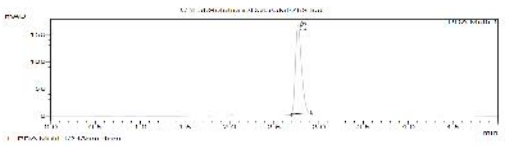

Fig 2: Chromatogram of Lenvatinib

Method Validation:

Linearity:

To establish the linearity of the method, standard solutions of Lenvatinib mesylate were prepared in the concentration range of 1–6 µg/mL. These were obtained by transferring 0.1 to 0.6 mL aliquots of a 100 µg/mL stock solution into a series of 10 mL volumetric flasks, followed by dilution to volume with methanol and thorough mixing. The absorbance of each solution was measured at 243 nm [13]. A calibration curve was constructed by plotting concentration (µg/mL) on the x-axis against the corresponding peak area on the y-axis. The results of method linearity was represented in table1.The method developed was achieved excellent linearity, with a correlation coefficient (R^2) of 0.999 as shown in figure 3 (Table 1).

Table 1: Results for Method Linearity

Concentration(µg/mL)	Peak area
1	529007
2	970312
3	1499781
4	1986883
5	2543139
6	3054841

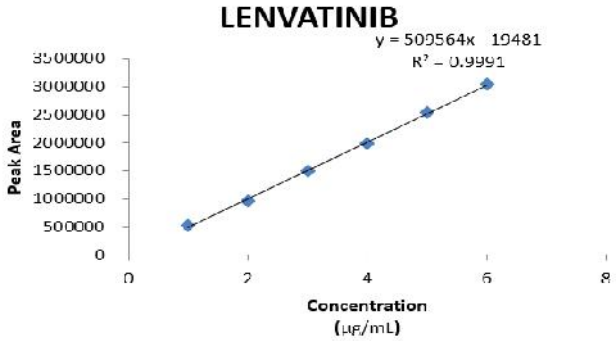

Fig 3: Calibration graph for Lenvatinib

Precision:

To assess precision, a stock solution of Lenvatinib mesylate was prepared by accurately weighing 10 mg of the working standard and transferring it into a 10 mL clean, dry volumetric flask. Approximately 5 mL of methanol (diluent) was added, and the solution was sonicated to ensure complete dissolution (Fig 3). The volume was then made up to the mark with the same solvent. From this stock solution, 0.1 mL was pipetted into a separate 10 mL volumetric flask and diluted to volume with methanol to obtain a final concentration of 1 µg/mL [14]. This solution was injected six times into the HPLC system under the optimized chromatographic conditions, and the peak areas were recorded to evaluate the method's precision as shown in Table 2.%RSD for the area of six standard injections results were less than 2% (Table 2).

Table 2: Results of Precision

	Intraday Precision		Interday Precision	
Injection No.	**RT(Min.)**	**Peak area**	**RT(Min.)**	**Peak area**
1	2.761	505129	2.766	505119
2	2.763	522461	2.773	522661
3	2.752	525946	2.758	525976
4	2.753	523268	2.752	521268
5	2.766	524355	2.763	524365
6	2.758	522681	2.763	522647
Mean		520640		520339.33
SD		7706.28		7631.70
%RSD		**1.48**		**1.46**

Accuracy:

Accuracy of the method was evaluated by recovery studies conducted at three concentration levels: 50%, 100%, and 150% of the target concentration. Standard solutions of Lenvatinib mesylate were spiked into the sample matrix and analyzed in triplicate. The mean peak area, observed concentration, and percentage recovery were calculated. The results demonstrated that the method provides accurate quantification of Lenvatinib mesylate, with recoveries ranging from 98.25% to 100.24% as shown in Table 3 [15].

Table 3: Accuracy results for LenvatinibMesylate

Accuracy	Peak area	Mean peak area	Concentration observed(µg/mL)	%Recovery
50%	986031	989777	1.98	99%
	991491			
	991811			
100%	1986784	1983183	3.93	98.25 %
	1985883			
	1976883			
150%	3044842	3045509	6.01	100.24 %
	3051841			
	3039844			

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ for Lenvatinib mesylate were calculated based on the standard deviation of the response (σ) and the slope (S) of the calibration curve, in accordance with ICH guidelines. The LOD and LOQ were calculated using the formulae LOD = 3.3σ/S, and LOQ = 10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Using a standard deviation (σ) of 953725 and a slope (S) of 509564, the LOD and LOQ were found to be 6.17 µg/mL and 18.7 µg/mL, respectively. These values indicate the method's sensitivity and its capability to detect and quantify low concentrations of Lenvatinib mesylate.

Robustness:

The robustness of the developed HPLC method was evaluated by making deliberate small variations in chromatographic conditions, including changes in flow rate (±0.1 mL/min) and detection wavelength (±2 nm). The effect of these variations was assessed by observing parameters such as retention time (RT), peak area, theoretical plate count, and tailing factor.

Under flow rate variations, the method showed consistent retention times ranging from 2.507 to 3.078 minutes, with peak areas varying slightly but remaining within acceptable limits. Theoretical plate counts remained above 5600 in all cases, and the tailing factor was consistently between 1.506 and 1.532, indicating good peak symmetry.

For wavelength changes, minor differences in peak areas were observed (2109830 at –2 nm and 2067440 at +2 nm), while plate counts (5877.78 and 5860.13, respectively) and tailing factors (1.513 and 1.514) remained stable. These results confirm that the method is robust against small changes in analytical conditions.

Assay:

An accurately weighed quantity of Lenvatinib mesylate, equivalent to 10 mg of active drug from a 62.5 mg tablet, was dissolved in 100 mL of methanol. From this solution, 0.1 mL was transferred into a 10 mL volumetric flask and diluted to volume with methanol. The prepared sample was analyzed using the validated HPLC method, the % assay was calculated and represented in Table 4.

Table 4: Results of assay

Drug	Label Claim(mg)	Average weight of Capsule(mg)	Mean peak area	%Assay
Lenvatinib mesylate	4	25	499812	98.75%

4. CONCLUSION

In this study, a robust and reliable HPLC method was developed and validated for the analysis of Lenvatinib mesylate in pharmaceutical formulations. The method demonstrated excellent linearity (R^2 = 0.999) across a concentration range of 1–6 µg/mL, with satisfactory precision, accuracy and sensitivity. The optimized chromatographic conditions, including the use of 100% methanol as the mobile phase and a detection wavelength of 243 nm, ensured consistent and clear separation with a retention time of 2.761 minutes. The method's accuracy was confirmed through recovery studies, with recoveries ranging from 98.25% to 100.24%. Additionally, the limits of detection and quantification (6.17 µg/mL and 18.7 µg/mL, respectively) indicate the method's sensitivity to low concentrations of Lenvatinib mesylate. The robustness of the method was also demonstrated through small variations in flow rate and wavelength, showing minimal impact on the analytical results. Finally, the method was successfully applied to determine the assay of Lenvatinib mesylate in a commercial tablet, yielding a result of 98.75%. The developed HPLC method for Lenvatinib mesylate is reliable, precise, and sensitive, making it ideal for routine quality control and analytical purposes in the pharmaceutical industry.

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