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Research Article

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A novel validated RP-HPLC-DAD method for the estimation of Lenvatinib Mesylate in bulk and pharmaceutical dosage form

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ABSTRACT

A novel approach was used to develop and validate a rapid, accurate, precise, simple, efficient and reproducible isocratic Reversed Phase-High Performance Liquid Chromatographic (RP-HPLC-DAD) method for the estimation of Lenvatinib Mesylate in bulk and pharmaceutical dosage form. Lenvatinib Mesylate were separated using Kromasil C_{18} column (250mm×4.6 mm, 5µm particle size), Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of 0.01M Ammonium acetate (pH adjusted to 3.5 with orthophosphoric acid) and Methanol (30:70, v/v). The flow rate was set to 1ml/min with the responses measured at 309nm. The retention time of Lenvatinib Mesylate was found to be 3.733min. Linearity was established for Lenvatinib Mesylate in the range of 10-125µg/ml with correlation coefficient (r^2=0.999). The percentage recoveries were between 100.3% to 100.6%. Validation parameters such as specificity, linearity, precision, accuracy, 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 developed method was successfully applied for the quantification and hyphenated instrumental analysis.

Keywords: Lenvatinib Mesylate, PDA detector, Hyphenated, ICH

INTRODUCTION

Lenvatinib Mesylate is a multiple receptor tyrosine kinase (RTK) inhibitor indicated for the treatment of thyroid cancer. Lenvatinib Mesylate 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 Mesylate is chemically known as 4- [3 - chloro – 4 - (cyclopropyl carbamoyl amino) phenoxy] - 7- methoxyquinoline – 6 – carboxamide; methane sulfonic acid was shown in Figure 1. Literature review reveals that very few analytical methods have been reported for the determination of Lenvatinib Mesylate which includes High performance liquid chromatography [2], Liquid chromatography-Mass spectroscopy [3-5] and Pharmacokinetics studies [6-8]. The present study was aimed to develop a novel, simple, economic and validated RP-HPLC-DAD method for the estimation of Lenvatinib Mesylate according to ICH guidelines [9].

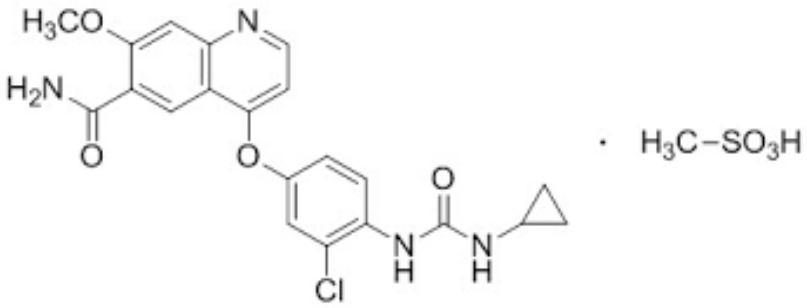

Figure 1: Chemical structure of Lenvatinib Mesylate

EXPERIMENTAL SECTION

Chemicals and Reagents:
Lenvatinib Mesylate bulk drug were kindly provided as gift sample by Manus Aktteva Biopharma LLP, Ahmedabad, India. Analytical grade of Ammonium acetate purchased from Rankem Ltd., India and HPLC grade of Methanol purchased from Merck Specialities Private Limited, India. HPLC grade of Water and Ortho phosphoric acid purchased from Rankem Ltd., India. Lenvima capsule contain Lenvatinib Mesylate 10mg is obtained from a local pharmacy manufactured by Eisai Pharmaceuticals India Pvt. Ltd, Mumbai, India.

Instrumentation:
The analysis was performed by using a chromatographic system from Waters Alliance e2695 HPLC system with 2998 PDA detector. The HPLC system was equipped with Empower 2 software. 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 conditions:
Lenvatinib Mesylate was analysed in Kromasil C_{18} (250mm×4.6 mm, 5µm particle size) column for the chromatographic separation. The mobile phase was composed of 0.01M Ammonium acetate (pH adjusted to 3.5 with orthophosphoric acid) and Methanol (30:70, v/v). Filtered through 0.45µm nylon membrane filter under vacuum filtration and pumped at ambient temperature, at a flow rate of 1 ml/min with UV detection wavelength at 309nm. Injection volume was 20µl. The run time was 5min and the retention time of Lenvatinib Mesylate was found to be 3.733min.

Chromatographic Parameters:

Equipment	: Waters Alliance e2695 HPLC system with 2998 PDA detector
Column	: Kromasil C_{18} (250mm×4.6 mm, 5µm particle size)
Flow rate	: 1ml/min
Wavelength	: 309 nm
Injection volume	: 20 µl
Column oven	: Ambient
Run time	: 5 Minutes

Preparation of Ammonium acetate buffer
A 0.01M Ammonium acetate buffer was prepared by dissolving 0.77gm of Ammonium acetate in 1000ml of HPLC grade water and pH was adjusted to 3.5 with orthophosphoric acid. The buffer was filtered through 0.45µm nylon membrane filter to remove all fine particles and gases.

Preparation of mobile phase:
The above prepared Ammonium acetate buffer and Methanol HPLC grade were mixed in the proportion of 30:70 v/v and was filtered through 0.45µm nylon membrane filter and degassed by sonication.

Preparation of diluent:
Mobile phase was used as diluent.

Preparation of standard stock solution of Lenvatinib Mesylate:
Standard stock solution of Lenvatinib Mesylate were prepared by dissolving 10mg of Lenvatinib Mesylate in 10ml of diluent into a 10ml clean dry volumetric flask and the standard solution was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Lenvatinib Mesylate.

Preparation of standard solution of Lenvatinib Mesylate for assay:
From the above standard stock solution of 1000µg/ml of Lenvatinib Mesylate further pipette 0.5 ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 50µg/ml of Lenvatinib Mesylate.

Selection of wavelength:
Standard stock solution of Lenvatinib Mesylate were prepared by dissolving 10mg of Lenvatinib Mesylate in 10ml of diluent into a 10ml clean dry volumetric flask and the standard solution was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Lenvatinib Mesylate. From the above standard stock solution of 1000µg/ml of Lenvatinib Mesylate further pipette 0.5 ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 50µg/ml of Lenvatinib Mesylate. The wavelength of maximum absorption (λmax) of 50µg/ml of Lenvatinib Mesylate were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The wavelength (λmax) was found to be 309nm shown in Figure 2.

Preparation of sample solution of Lenvatinib Mesylate:
Twenty capsules were accurately weighed and capsule powder equivalent to 10mg of Lenvatinib Mesylate were taken into 10ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent and filtered through 0.45 µm nylon membrane filter. Further pipette out 0.5ml from the above Lenvatinib Mesylate sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 50µg/ml of Lenvatinib Mesylate. 20µL from standard and sample solution were injected into the chromatographic system and the peak areas were measured for Lenvatinib Mesylate was shown in Figure 3 and 4 respectively. The % Assay was calculated by comparing the peak area of standard and sample chromatogram by using the formula given below and the assay result was shown in Table 1.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:
AT = Average peak area of sample preparation
AS= Average peak area of standard preparation
WS = Weight of standard taken in mg
WT=Weight of sample taken in mg
P = Percentage purity of working standard
DS= Dilution factor for standard preparation
DT=Dilution factor for sample preparation

Validation of the proposed method
The developed method for the simultaneous estimation of Lenvatinib Mesylate was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ) [10].

System Suitability:
At first the HPLC system was optimized as per the chromatographic conditions. One blank followed by six replicates of a single calibration standard solution of 50µg/ml of Lenvatinib Mesylate was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates and peak asymmetry were taken and results were presented in Table 2.

Specificity:
The effect of excipients and other additives usually present in the dosage form of Lenvatinib Mesylate in the determination under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the blank and placebo solution into the HPLC system. The representative chromatogram of blank and placebo was shown in Figure 5 and 6 respectively.

Linearity for Lenvatinib Mesylate:
Aliquots of 0.1, 0.25, 0.5, 0.75, 1 and 1.25ml of standard working solution of Lenvatinib Mesylate was pipetted out from the standard stock solution of 1000µg/ml of Lenvatinib Mesylate and transferred into a series of 10ml clean dry volumetric flask and make volume up to the mark with the same diluent to get the concentration of 10, 25, 50, 75, 100 and 125µg/ml of Lenvatinib Mesylate. The calibration standard solutions of Lenvatinib Mesylate were injected using a 20µl Hamilton Rheodyne injector and the chromatograms were recorded at 309nm and a calibration graph was obtained by plotting peak area versus concentration of Lenvatinib Mesylate. The linearity data is presented in Figure 7 and Table 3. Acceptance Criteria: Correlation coefficient should be not less than 0.999

Accuracy studies for Lenvatinib Mesylate:
The accuracy of the method was determined by calculating recovery of Lenvatinib Mesylate by the method of standard addition. Known amount of standard solution of Lenvatinib Mesylate at 50%, 100% and 150% was added to a pre quantified sample solution and injected into the HPLC system. The mean percentage recovery of Lenvatinib Mesylate at each level was calculated and the results were presented in Table 4.

Preparation of pre quantified sample solution for accuracy studies:
Twenty capsules were accurately weighed and capsule powder equivalent to 10mg of Lenvatinib Mesylate were taken into 10ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent and filtered through 0.45 µm nylon membrane filter. Further pipette out 0.5ml from the above Lenvatinib Mesylate sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 50µg/ml of Lenvatinib Mesylate.

Preparation of standard solution of Lenvatinib Mesylate for accuracy studies:
Standard stock solution of Lenvatinib Mesylate were prepared by dissolving 10mg of Lenvatinib Mesylate in10ml of diluent into a 10ml clean dry volumetric flask and the standard solution was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Lenvatinib Mesylate.

Preparation of 50% standard solution:
From the standard stock solution of 1000µg/ml of Lenvatinib Mesylate further pipette 0.25ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 25µg/ml of Lenvatinib Mesylate.

Preparation of 100% standard solution:
From the standard stock solution of 1000µg/ml of Lenvatinib Mesylate further pipette 0.5ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 50µg/ml of Lenvatinib Mesylate.

Preparation of 150% standard solution:
From the standard stock solution of 1000µg/ml of Lenvatinib Mesylate further pipette 0.75ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 75µg/ml of Lenvatinib Mesylate.

Acceptance Criteria**:** The % Recovery for each level should be between 98.0 to 102.0%.

Precision studies for Lenvatinib Mesylate:
Method precision (Repeatability):
Twenty capsules were accurately weighed and capsule powder equivalent to 10mg of Lenvatinib Mesylate were taken into 10ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent and filtered through 0.45 µm nylon membrane filter. Further pipette out 0.5ml from the above Lenvatinib Mesylate sample stock solution into a 10ml volumetric flask and diluted up to

the mark with diluent to get the concentration of 50µg/ml of Lenvatinib Mesylate. A homogenous sample of a single batch analysed six times and was checked whether the method is giving consistent results. The %RSD for the area of six replicate injections was calculated as mentioned in Table 5.

Acceptance Criteria: The % RSD for the peak area of six sample injections should not be more than 2%.

System precision:
The system precision was carried out to ensure that the analytical system is working properly. The standard preparation concentration of 50µg/ml of Lenvatinib Mesylate was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in Table 6.

Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Intermediate precision/ruggedness:
The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on different laboratory by different analyst and different days. The sample preparation concentration of 50µg/ml of Lenvatinib Mesylate was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in Table 7.

Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ):
Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD of Lenvatinib Mesylate was calculated and shown in Table 8. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Lenvatinib Mesylate was calculated and shown in Table 8.

Robustness:
As part of the Robustness, deliberate change in the flow rate and mobile phase proportion of ±10% was made to evaluate the impact on the method. The results reveal that the method is robust. The results are summarized in Table 9 and 10.

Stability of solution:
The %RSD of the assay of Lenvatinib Mesylate from the solution stability and mobile phase stability experiments was within 2%. The results of the solution and mobile phase stability experiments confirm that the sample solutions and mobile phase used during the assays were stable upto 48hours at room temperature was calculated and shown in Table 11.

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Lenvatinib Mesylate were obtained with a mobile phase containing a mixture of 0.01M Ammonium acetate buffer (pH adjusted to 3.5 with orthophosphoric acid) and Methanol (30:70, v/v) was delivered at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 309nm based on peak area. The retention time of Lenvatinib Mesylate was found to be 3.733min. Linearity was established for Lenvatinib Mesylate in the range of 10-125µg/ml with correlation coefficient 0.999 and mean accuracies were found to be is 100.03% to 100.06% for Lenvatinib Mesylate, which indicates accuracy of the proposed method. The % RSD values of accuracy for Lenvatinib Mesylate were found to be < 2 %. The % RSD value of method precision was 0.19% for Lenvatinib Mesylate and % RSD value of system precision was 0.54% for Lenvatinib Mesylate. The % RSD value of reproducibility is 0.06% for Lenvatinib Mesylate reveal that the proposed method is precise. LOD value for Lenvatinib Mesylate was found to be 1.2µg/ml and LOQ value for Lenvatinib Mesylate were found to be 3.8µg/ml. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough. These data show that the proposed method is specific and sensitive for the determination of Lenvatinib Mesylate.

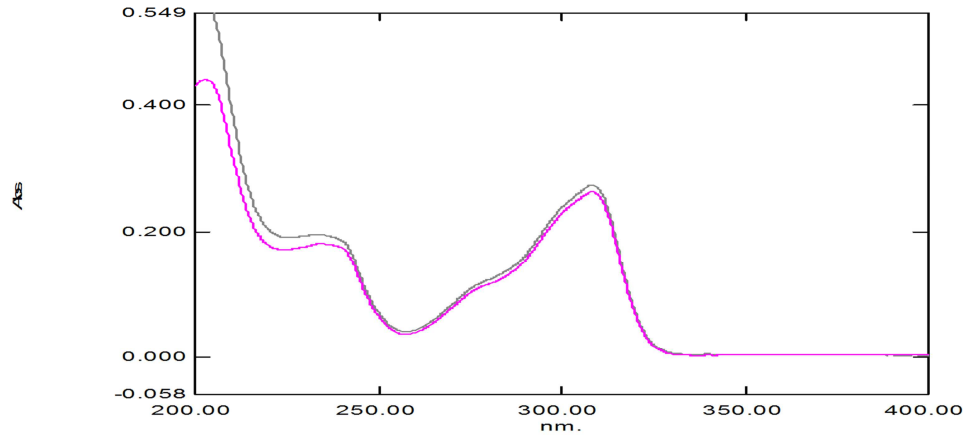

Figure 2: UV-Spectrum of Lenvatinib Mesylate at 309nm

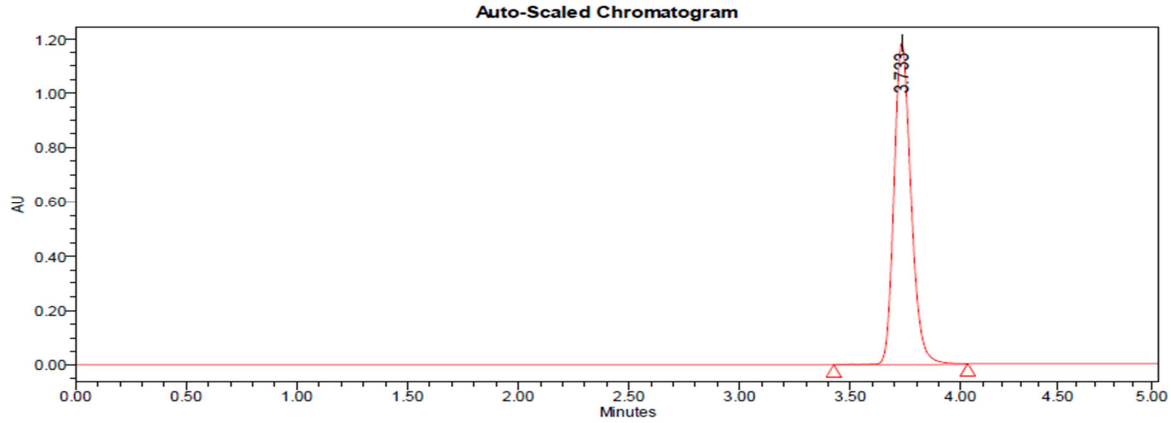

Figure 3: Standard chromatogram of Lenvatinib Mesylate

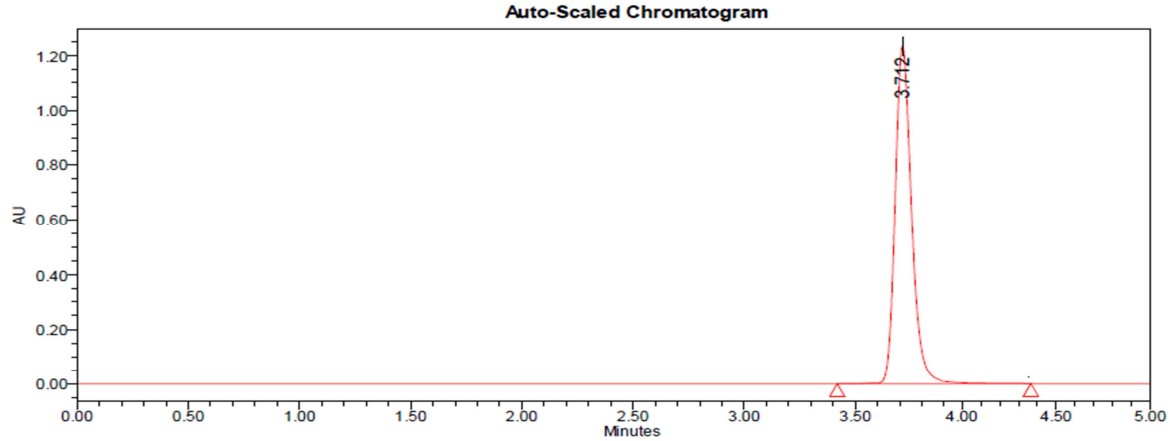

Figure 4: Sample chromatogram of Lenvatinib Mesylate

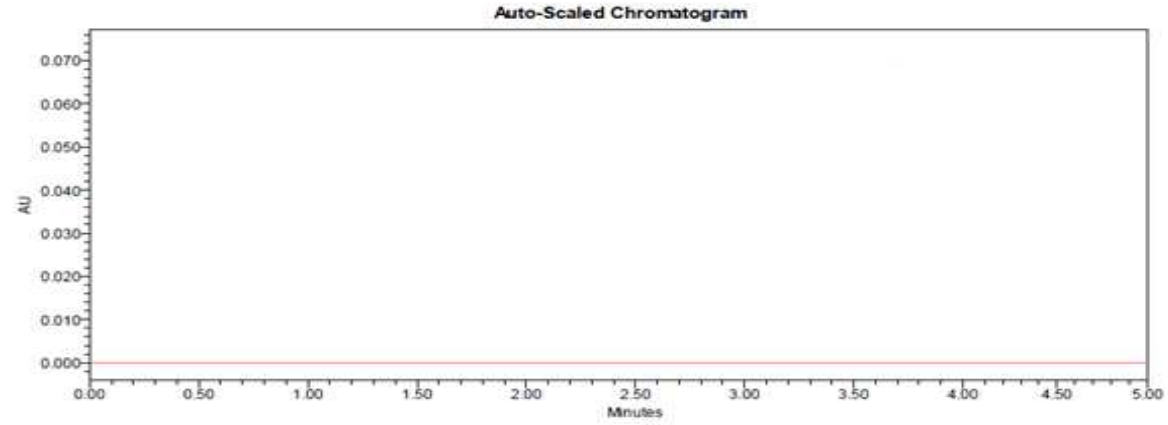

Figure 5: Chromatogram of Blank

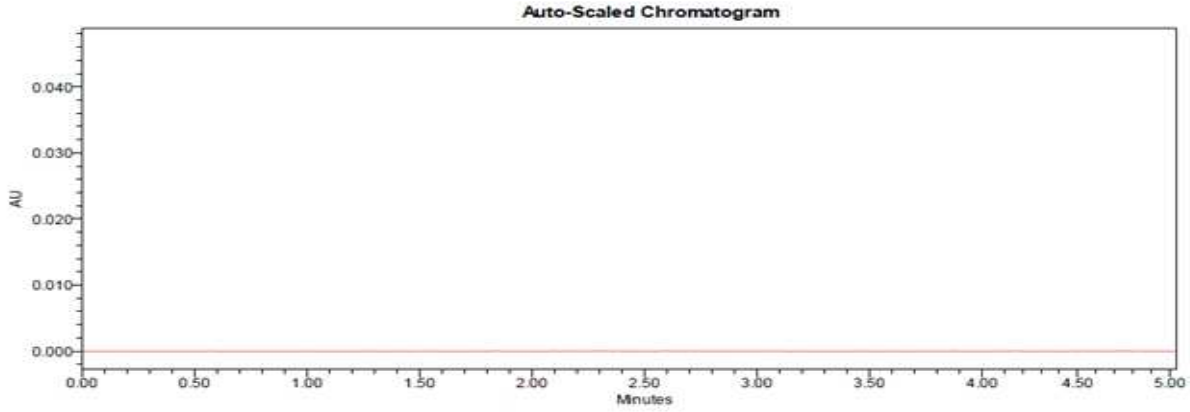

Figure 6: Chromatogram of Placebo

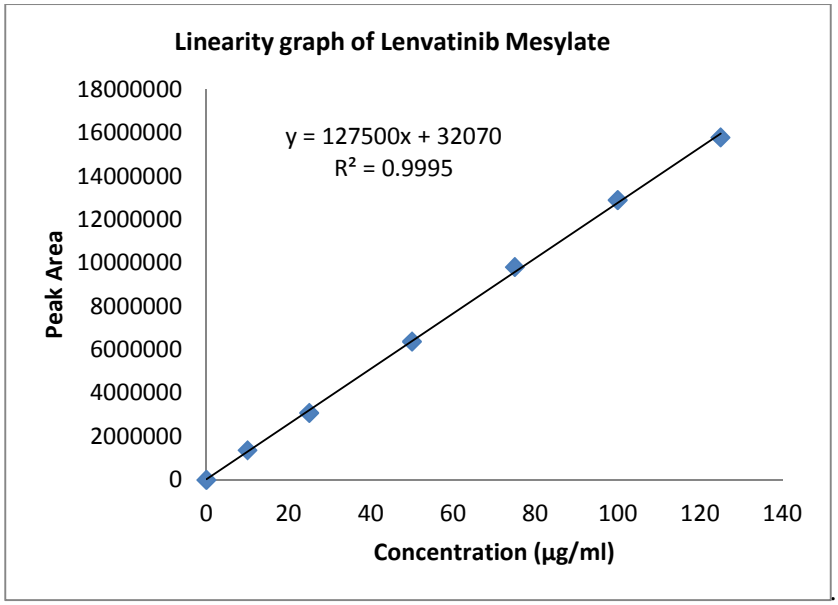

Figure 7: Linearity graph of Lenvatinib Mesylate

Table 1: Assay of marketed formulation of Lenvatinib Mesylate

Drug	Lenvima Capsule Label Claim (mg)	Amount Found (mg)	% Label Claim ± % RSD (n=3)
Lenvatinib Mesylate	10	9.986	99.86±0.31

Table 2: System suitability test parameters for Lenvatinib Mesylate

Parameter (n=6)	Lenvatinib Mesylate
Retention Time (Mins)	3.733
Theoretical plates	2633
Tailing factor	1.2

Table 3: Linearity data for Lenvatinib Mesylate

Linearity of Lenvatinib Mesylate	
Concentration (µg/ml)	**Peak Area**
10	1361098
25	3086279
50	6375650
75	9806576
100	12897238
125	15785103

Table 4: Recovery study data of Lenvatinib Mesylate

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S_1:50%	25	25.01	100.04	Mean-100.03
S_2:50%	25	24.98	99.92	S.D-0.10
S_3:50%	25	25.03	100.12	%RSD-0.10
S_4:100%	50	50.03	100.06	Mean-100.06
S_5:100%	50	50.05	100.09	S.D-0.03
S_6:100%	50	50.02	100.03	%RSD=0.03
S_7:150%	75	74.92	99.89	Mean-100.05
S_8:150%	75	75.13	100.18	S.D-0.15
S_9 :150%	75	75.06	100.08	%RSD-0.15

Table 5: Method precision data for Lenvatinib Mesylate

Lenvatinib Mesylate				
S.No.	Concentration (µg/ml)	Retention time (min)	Peak Area	% Assay
1	50	3.701	6439408	100.44
2	50	3.700	6421152	100.15
3	50	3.703	6416074	100.07
4	50	3.701	6412284	100.02
5	50	3.704	6435171	100.37
6	50	3.705	6440083	100.45
Average		3.702	6427362	100.25
SD		0.001966	12338.88	0.192455
%RSD		0.05	0.19	0.19

TABLE 6: System precision data for Lenvatinib Mesylate

Lenvatinib Mesylate			
S.No.	Concentration (µg/ml)	Retention time (min)	Peak Area
1	50	3.694	6381099
2	50	3.697	6360585
3	50	3.698	6346112
4	50	3.697	6384204
5	50	3.701	6301709
6	50	3.701	6315208
Average		3.698	6348153
SD		0.002683	34018.38
%RSD		0.07	0.54

TABLE 7: Ruggedness data for Lenvatinib Mesylate

Ruggedness Data for Lenvatinib Mesylate								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Conc. (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
50	99.65	100.16	100.13	100.15	100.13	100.16	100.14	100.02
50	99.57	99.71	99.84	100.28	99.84	100.08	100.01	99.84
50	99.51	100.15	99.76	99.92	99.92	99.99	99.22	100.03
50	99.60	100.11	100.02	100.01	99.98	100.14	99.70	99.72
50	99.21	100.12	99.98	100.15	100.17	100.17	99.90	100.19
50	99.25	99.98	100.14	100.12	100.15	100.14	100.03	100.01
Average	99.47	100.04	99.98	100.11	100.03	100.11	99.83	99.97
SD	0.1887	0.1722	0.1511	0.1253	0.1368	0.0695	0.3364	0.1635
%RSD	0.19	0.17	0.15	0.13	0.14	0.07	0.34	0.16
Intermediate precision within-laboratories variations (n=24)								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Average	99.90				**Average**	99.99		
SD	0.291				**SD**	0.118		
%RSD	0.3				**%RSD**	0.1		
Reproducibility between laboratories (n=48) (% Assay)								
Average	99.95							
SD	0.064							
%RSD	0.06							

Table 8: Summary of validation parameter for Lenvatinib Mesylate

Parameters	**RP-HPLC method**	
	Lenvatinib Mesylate	
Linearity range (µg/ml)	10-125	
Slope	12750	
Intercept	32070	
Correlation coefficient	0.999	
LOD (µg/ml)	1.2	
LOQ (µg/ml)	3.8	
Method Precision (% RSD, n=6)	0.19	
System precision (% RSD, n=6)	0.54	
Ruggedness (% RSD, n=24)	Lab-1	Lab-2
	0.3	0.1
Reproducibility (% RSD, n=48)	0.06	
% Accuracy	100.03-100.06	
Robustness (% RSD, n=6)	Less Flow rate	More Flow rate
	0.18	0.36
	Less Organic phase	More Organic phase
	0.05	0.08

Table 9: Summary of Robustness (Change in Flow Rate) for Lenvatinib Mesylate

Drug	Change in Flow rate (ml/min)	Retention Time (Mins)	Robustness (0.9 ml/min to 1.1 ml/min)				
			Average peak area (n=6)	SD	% RSD	USP Plate Count	Asymmetry
Lenvatinib Mesylate	0.9	4.152	6832590	12109.48	0.18	2964	1.1
	1.0	3.733	6375910	7092.138	0.11	2633	1.2
	1.1	3.626	6267369	22509.22	0.36	2826	1.1

Table 10: Summary of Robustness (Change in Mobile Phase) for Lenvatinib Mesylate

Drug	Change in Mobile Phase	Retention Time (Mins)	Change in mobile phase (0.01M Ammonium acetate (pH adjusted to 3.5 with orthophosphoric acid) and Methanol) (37:63 v/v to 23:77v/v)				
			Average peak area (n=6)	SD	% RSD	USP Plate Count	Asymmetry
Lenvatinib Mesylate	10% less Organic (37:63 v/v)	3.481	6193058	3049.594	0.05	2971	1.2
	Actual (30:70 v/v)	3.733	6375910	7092.138	0.11	2633	1.2
	10% more Organic (23:77 v/v)	4.363	6172936	5136.165	0.08	2479	1.1

Table 11: Summary of solution stability-effect of P^H of mobile phase (0.01M Ammonium acetate buffer and Methanol (30:70, v/v) (P^H adjusted to 3.5 with orthophosphoric acid) for Lenvatinib Mesylate for 48 hours at room temperature

	Solution stability for Lenvatinib Mesylate					
S.No.	Concentration (µg/ml)	Retention time (min)	Peak Area	%Assay	USP Plate Count	Asymmetry
1	50	3.712	6376523	99.46	2735	1.12
2	50	3.711	6387800	99.63	2745	1.1
3	50	3.711	6356236	99.14	2777	1.1
4	50	3.712	6353348	99.10	2776	1.12
5	50	3.718	6348984	99.03	2717	1.1
6	50	3.718	6348398	99.02	2761	1.1
Average		3.714	6361882	99.23	2752	1.11
SD		0.003386	16364.5	0.25525	23.85302	0.010328
%RSD		0.09	0.26	0.26	0.87	0.93

CONCLUSION

RP-HPLC method for the estimation of Lenvatinib Mesylate in their bulk and pharmaceutical dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Lenvatinib Mesylate in the range of 10-125µg/ml with correlation coefficient 0.999. The percentage recovery of drug was achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Lenvatinib Mesylate in their bulk and pharmaceutical dosage form.

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