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Stability Indicating UPLC Method Development and Validation of Lenvatinib

Sai Prasanna A*, Meruva Sathish Kumar, R. V. Valli Kumari and S. Marakatham.

Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda Dhulapally, Hyderabad - 500014.

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***Corresponding Author Email:** prasannagupta002@gmail.com

Abstract

A new precise accurate, rapid method has been developed for the estimation of lenvatinib pharmaceutical dosage form by UPLC. From results the proposed method if highly sensitive, précised and accurate and it successfully applied for the quantification of API content in commercial formulations of Lenvatinib Educational Institutions and Quality Control Laboratories. A simple and selective UPLC method is described for the determination of lenvatinib & Chromatographic separation was achieved on a Acquity BEH C18 (50*3.00mm. 1.7µm) using mobile phase consisting of 0.1% ortho-phosphoric acid: Acetonitrile (60:40) v/v was pumped with flow rate of 0.5ml/min with isocratic elution mode and optimised wavelength was 248nm. Linearity was observed in the range 30-70µg/ml for lenvatinib (r^2 =0.994). Accuracy was also performed at 50, 100, 150% recovery and the result obtained was 100.8 and it is within the range 98-102%. Robustness was performed with 2 different parameters by changing the flow rate and temperature and tailing factor obtained was within the limits. Different degradation studies like peroxide degradation, photolytic degradation, Acid degradation, Alkaline degradation, Thermal degradation studies were performed. Validation parameters performed were in good agreement with the acceptance limits.

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INTRODUCTION

LENVIMA is kinase inhibitor, is the mesylate salt of Lenvatinib. Its chemical name is 4-{3-chloro-4-[(cyclopropyl carbamoyl)amino] phenoxy}-7-methoxy quinoline-6-carboxamide. The molecular formula is $C_{22}H_{19}ClN_4O_4$ and its molecular weight is 426.86. It is practically insoluble in water and sparingly soluble in methanol. It has the pKa value of 5.05 and the melting point is 221-224°C. It is available in the form of capsule for oral use.

Lenvatinib is a receptor tyrosine kinase (RTK) inhibitor that inhibits the kinase activities of vascular endothelial growth factor (VEGF) receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4). Lenvatinib also inhibits other RTKs that have been implicated in pathogenic angiogenesis, tumour growth, and cancer progression in addition to their normal cellular functions, including fibroblast growth factor (FGF) receptors FGFR1, 2, 3, and 4; the platelet derived growth factor receptor alpha (PDGFRα), KIT, and RET. These receptor tyrosine kinases (RTKs) located in the cell membrane play a central role in the activation of signal transduction pathways involved in the normal regulation of cellular processes, such as cell proliferation, migration, apoptosis and differentiation, and in pathogenic angiogenesis,

lymphogenesis, tumour growth and cancer progression. In particular, VEGF has been identified as a crucial regulator of both physiologic and pathologic angiogenesis and increased expression of VEGF is associated with a poor prognosis in many types of cancers. It is used in the treatment of thyroid cancer.

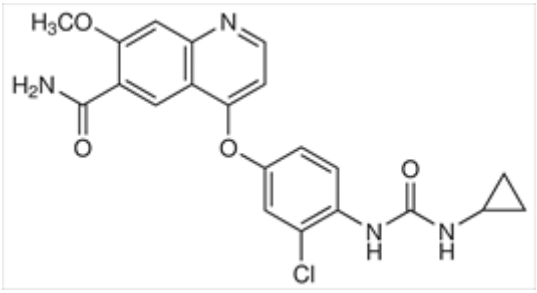

Fig. 1 Chemical structure of Lenvatinib

The main aim of the in process investigation is to develop an accurate, precise, sensitive, reproducible method development and validation of Lenvatinib.

The Objectives to develop analytical method:

- Selecting the UPLC separation mode
- Optimising mobile phase
- Selection of column for analysis
- Selection of the appropriate detector
- Selection of mode of elution of sample
- Selection of flow rate, temperature and pH

MATERIALS & METHODS

The following instuments and materials were used either Rankem/AR grade or the best possible pharma grade available as supplied by the manufacture or supplier without further purification or investigation.
Drug Samples: Were obtained from Chandra pharma Laboratories pvt Ltd.

Chemicals and Solvents used

Potassium Di-hydrogen-ortho phosphate & Dipotassium hydrogen orthophosphate - Rankem/ AR Grade
Acetonitrile- Merck/ HPLC Grade
Water- Merck/ HPLC Grade
Methanol- Merck/ HPLC Grade
O-Phosphoric acid- Rankem/ AR Grade
Lenvatinib (LENVIMA-10mg) Capsules

Preparation of Standards and sample:

Preparation of 0.1% ortho phosphoric acid:

Taken 1mL of ortho-phosphoric acid and transferred in to a 1000mL of water & filtered through 0.45µm filters to remove all fine particles and gases.

Preparation of Standard solution

Accurately Weighed about 100 mg of Lenvatinib & transferred in to a 100mL volumetric flask, then added 70mL of diluent, sonicated for 3min. Made final volume up to mark with the diluents &mixed well(1000µgm/ml).
Taken 5mL of standard stock solution and transferred in to 50mL volumetric flask then diluted up to mark with diluents & mixed well (100µg/ml).

Preparation of Working Standard solution

From above standard solution 1ml was transferred to 10mL volumetric flask then diluted up to mark with diluents & mixed well (10µg/ml).

Preparation of samples

Diluent: Mobile phase used as Diluent

Preparation of Sample solution

Sample name: LENVIMA CAPSULES 10mg
Weigh 20capsules by removing the shell then crush with mortar and pestle then weigh a quantity of powder equivalent to 100mg of Lenvatinib and transferred in to a 100mL volumetric flask, then added 70mL of diluent, sonicated for 30min. Made final volume up to mark with the diluent & mixed well. (1000µgm/ml)
Taken 5mL of sample solution and transferred in to 50mL volumetric flask then diluted up to mark with diluent & mixed well, filter this final solution through 0.45µm PVDF Syringe filter (100µgm/ml).

RESULTS AND DISCUSSIONS

Solubility Studies

These studies are carried out at 25 ^{0}C

Table 1: Solubility studies.

Solvent Name	Lenvatinib Mesylate
Water	Sparingly Soluble
Methanol	Soluble
Ethanol	Soluble

Determination of Working Wavelength (λ_{max})

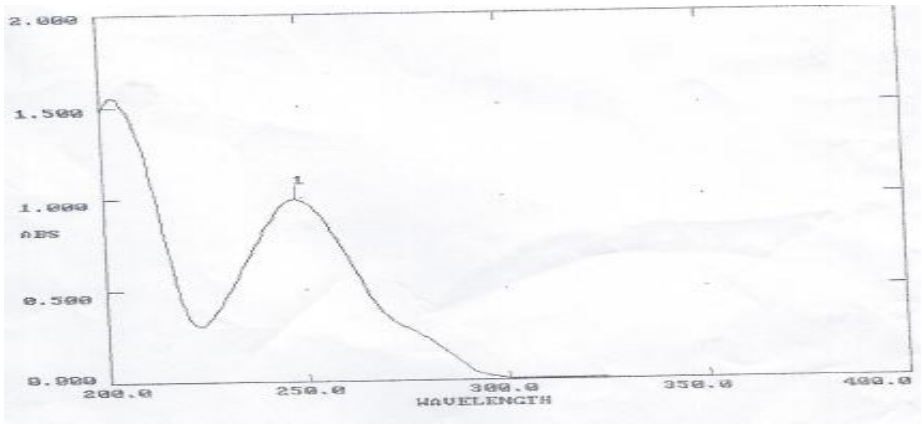

Fig. 2: UV-VIS Spectrum of Lenvatinib Mesylate (248 nm)

Trial -1

Chromatographic conditions

Column	:	Zorbax cyano (50x2.1mm ID) 1.8µm
Elution mode	:	Isocratic
Mobile phase	:	Methanol: water [50:50]
Flow rate	:	0.5mL/min
Detection wavelength	:	248 nm
Injection volume	:	10µL
Run time	:	15 min

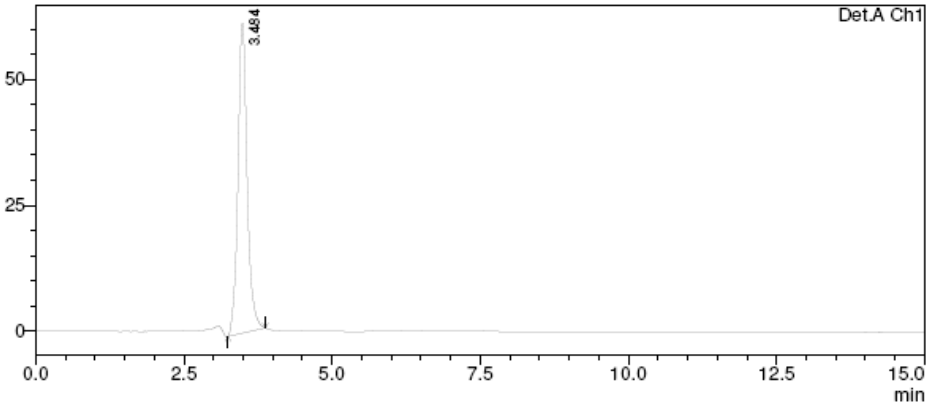

Fig 3: Chromatogram of Trail 1

Table no 2: Results for Trail 1

S.NO	Name	RT	Area	TP	TF	Rs
1	Lenvatinib	3.484	115637	1085	1.95	-

Observation
Peak shape was not good and efficiency was not within the acceptance criteria. So this trial was not considered.

Optimised trial Chromatographic conditions
Column : Acquity BEH C18 (50*3.0mm. 1.7µm)
Elution mode: Isocratic
Mobile phase : 0.1% Orthophosphoric acid: Acetonitrile (60:40) v/v
Flow rate : 0.5mL/min
Detection wavelength : 248nm
Injection volume : 10µL
Run time : 5min
Standard solution is used for recording chromatogram

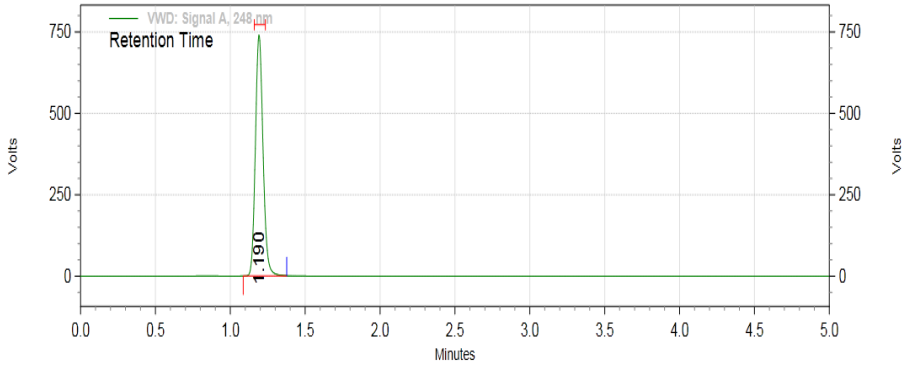

***Fig.4: Chromatogram* of Optimised trial**

***Table 3*: Results for Optimised Trial**

S.NO	Name	RT	Area	TP	TF
1	Lenvatinib	1.190	44113817	2652	1.2

Observation: From the above trial Lenvatinib eluted with good peak shape. The Theoretical plates & tailing factor ware found to be within limits. So this trail was considered and validated according to ICH guidelines.

CHROMATOGRAMS OF ASSAY (STANDARD & SAMPLE):
The standard & sample preparations were prepared and injected six times and area and chromatograms were recorded.

Table 4: Results for Assay of Lenvatinib

Lenvatinib		
	Standard Area	**Sample Area**
Injection-1	44049957	43312224
Injection-2	44176366	43309599
Injection-3	43965547	43398270
Injection-4	44027772	44329833
Injection-5	43915825	44358634
Average Area	44027093.4	43741712
Standard deviation	551274.21	
%RSD	0.22	
Assay(%purity)	99.35	

Table 5: Results of assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
LENVATINIB	10	9.87	98.7

Observation

So the % assay found to be within the limits that is 95.0-105.0% as per IP.

VALIDATION:

System Suitability& System precision:

To verify the system suitability & system precision 100µgm/ml of sample solution was taken and injected six times and chromatograms were recorded.

Table no 6: Results for system suitability of LENVATINIB.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.192	44125114	2560	1.2
2	1.193	44176891	2421	1.1
3	1.193	44165637	2676	1.3
4	1.190	44147346	2706	1.2
5	1.187	44105682	2704	1.1
6	1.190	44102411	2786	1.2
Mean	-	44137180	-	-
SD	0.023	31102.11	-	-
%RSD	0.2	0.1	-	-

Acceptance criteria

1. The % RSD for the retention time of LENVATINIB Peaks from 6 replicate injections of each Standard solution should be not more than 2.0
2. The % RSD for the peak area responses of LENVATINIB peak from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the LENVATINIB peaks is not less than 2000.
4. The Tailing factor (TP) for the LENVATINIB peak is not more than 2.0.

Observation:

The plate count and tailing factor results were found to be within the limits and The % RSD was found to be 0.1 so system is suitable and giving precise results

Method precision

The samples of 100µg/ml were injected six times separately and areas were noted.

The chromatograms were recorded and the results were summarized in Table

Injection	LENVATINIB	
	Area	% Assay
1	44113817	98.9
2	44176366	98.7
3	44078346	98.4
4	44150181	98.7
5	44008775	98.9
6	44025521	98.0
Average		9666992
SD		2938.097
%RSD		0.030383

Observation

The %RSD of Assay for 6 Samples determinations of LENVATINIB found to be within the acceptance criteria (less than 2.0%). %Assay Also within the limits (95.0 to 105.0) hence method is precise.

Linearity and range

Standard stock solutions of Lenvatinib were prepared by dissolving 100 mg of Lenvatinib in 100mL of diluent. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min further dilutions were done. The different concentrations of 60,80,100,120,140µgm/ml were prepared and area was plotted. The correlation coefficient R^2 was determined.

A graph was plotted against concentrations & area obtained

Table no 7: Linearity data of LENVATINIB.

S.No	Concentration (µg/mL)	Area
1	60	21720461
2	80	34167231
3	100	44035624
4	120	52943892
5	140	67035271

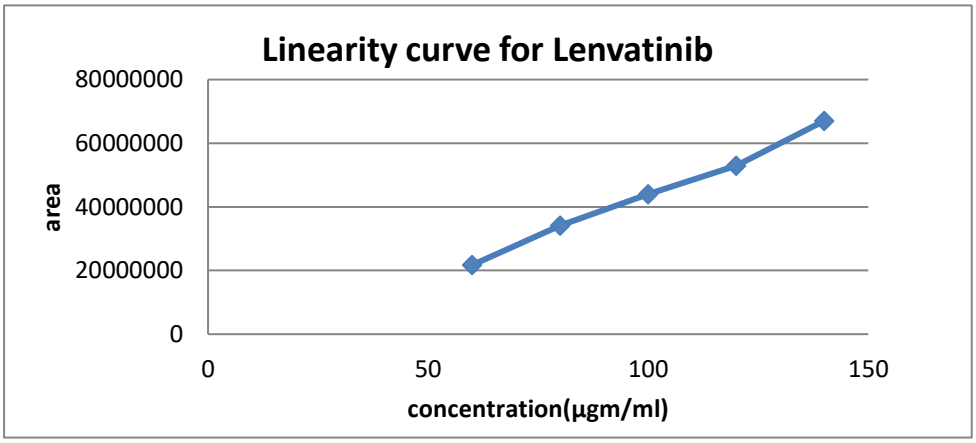

Fig 5: Graph for Linearity data of LENVATINIB.

Table 8: Linearity results of LENVATINIB.

S.No	Parameter	LENVATINIB
1	Correlation coefficient	0.994
2	Slope	51769
3	Intercept	54703

Acceptance criteria

The relationship between the concentration (in %) of LENVATINIB and area of LENVATINIB should be linear in the specified range and the correlation should not be less than 0.9999.

Observation:

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparation was found to be 0.994

Specificity:

Blank solution and placebo solution were prepared and chromatogram was plotted.

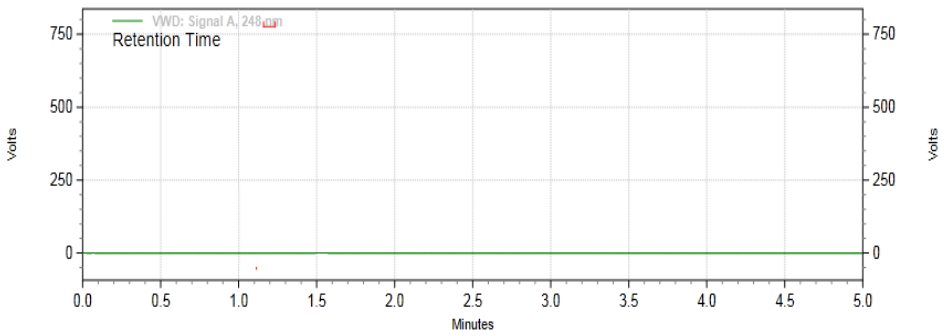

Fig 6: Chromatogram of Placebo

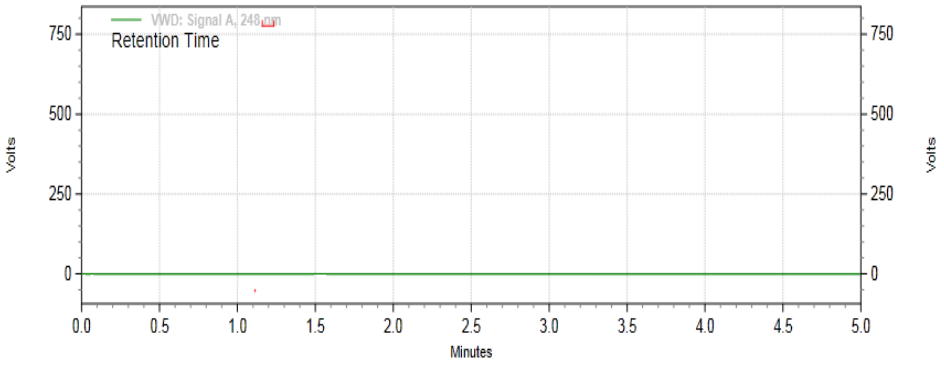

Fig 7: Chromatogram of Blank

Observation: Chromatograms of blank and placebo solutions had shown no peaks at the retention times of Lenvatinib. It was observed that diluent or excipient peaks do not interfere with the LENVATINIB Peak.

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs **(50µg/ml, 100µg/ml and 150µg/ml)** were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

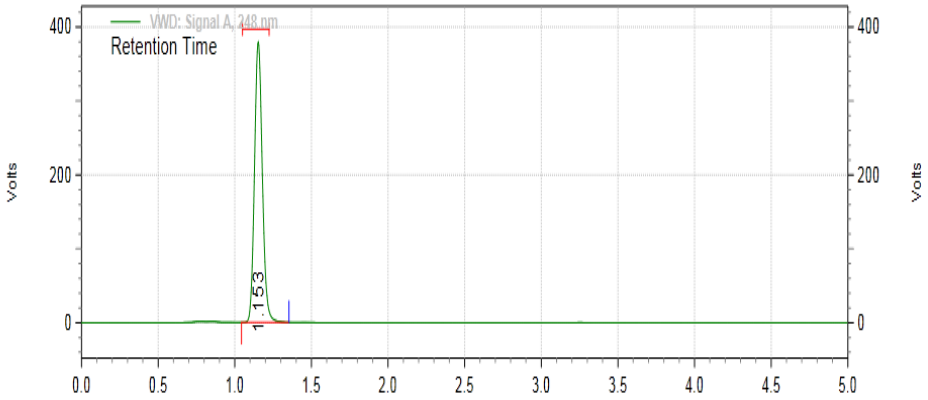

Fig 8: Chromatogram of 50% recovery-1

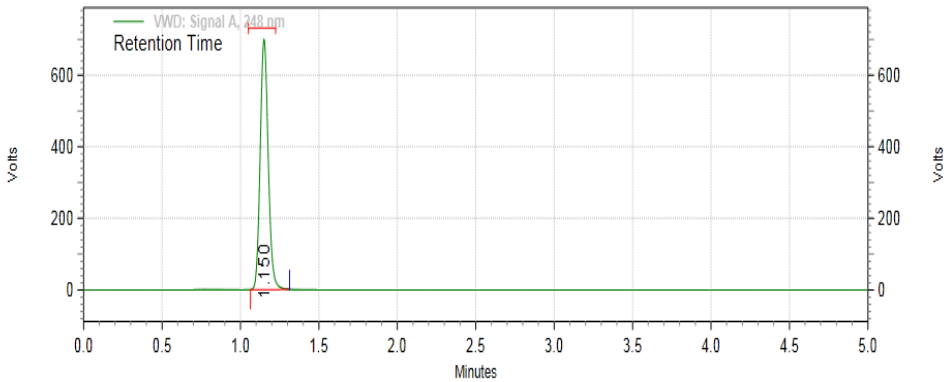

Fig 9: Chromatogram of 100% recovery-1

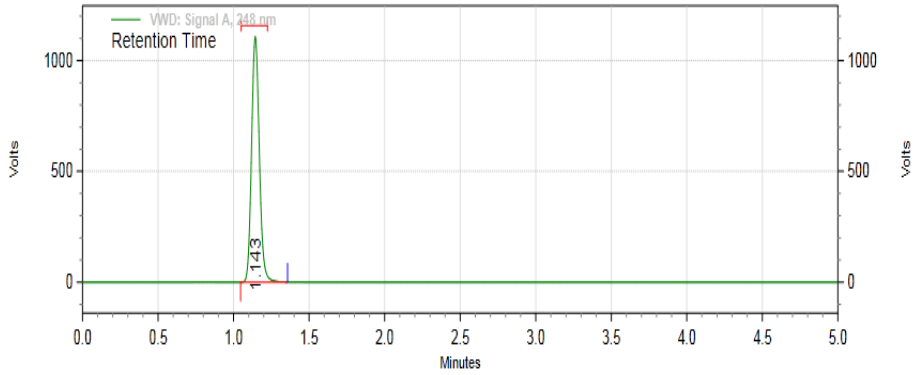

Fig 10: Chromatogram of 150% Recovery-1

Table no 9: Accuracy results of lenvatinib

LENVATINIB					
Name of the sample	**Standard weight in (mg)**	**Area**	**Conc Recovered(µg/ml)**	**% Recovery**	**Average**
50% Recover_01	51.25	22823459	51.84	98.9	
50% Recover_02	51.36	22610539	51.36	100.0	
50% Recover_03	51.24	22323998	50.71	101.1	
100% Recovery_01	97.21	41807632	94.96	102.4	
100% Recovery_02	97.25	41742541	94.81	102.6	100.8
100% Recovery_03	97.36	41743350	94.81	102.7	
150% Recovery_01	150.21	66362511	150.73	99.7	
150% Recovery_02	150.89	66368811	150.75	100.1	
150% Recovery_03	150.22	66320623	150.64	99.7	

Acceptance criteria The Average % recovery of Lenvatinib should lie between 98% and 102%.
Observation: The percentage mean recovery of Lenvatinib was found between 98.0 to 102.0%
Robustness

The sample solution (100µgm/ml) was taken and robustness was determined by change in the flow rate such as0.4-0.5ml/min, 0.5-0.6ml/min and also temperature between 25-30°C and 30-35°C.

Table 10: Results for Robustness of LENVATINIB

Chromatographic changes		Retention time(min)	Tailing Factor	Theoretical Plates
Flow rate (mL/min)	0.4	1.473	1.1	2942
	0.6	0.933	1.2	2047
Temperature (°C)	25	1.143	1.2	2467
	35	1.137	1.1	2496

Observation: The tailing factor was found to be within the limits on small variation of flow rate and temperature.

Ruggedness The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Table no 11: Ruggedness Results of LENVATINIB

LENVATINIB	%Assay	LENVATINIB	%Assay
Analyst 01	98.8	Analyst 01	98.9
Analyst 02	98.9	Analyst 02	98.1
%RSD	0.18	%RSD	0.20

Acceptance criteria: The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Observation: From the above results % Assay and %RSD obtained acceptance criteria 2% so method is rugged

STABILITY STUDIES

Table no 12: Observation of degradation of drug in degradation studies

Method	Std area	Degradation area	% Obtained	% Degraded
Peroxide	44027093.4	43361348	98.487	0.213
Photolytic	44027093.4	43327162	98.410	0.290
Acidic	44027093.4	43320002	98.393	0.307
Alkaline	44027093.4	43311537	98.374	0.326
Thermal	44027093.4	43352114	98.466	0.234

Acceptance criteria: The % Degraded for LENVATINIB from these stability methods should be not more than
1.0 %.
Result: All the degradation studies performed are under the acceptance criteria

CONCLUSION

A new precise, accurate, rapid method has been developed for the estimation of Lenvatinib pharmaceutical dosage form by UPLC.
The optimum wavelength of lenvatinib was found to be 248nm. Various trials were performed using different mobile phases in different ratios but finally 0.1% orthophosphoric acid & acetonitrile were selected as good peak was obtained. Retention time was found to be 1.190min.
Different analytical procedures such as linearity, precision, accuracy, specificity were performed according to International Conference on Harmonisation ICH Q2B guidelines. From the linearity correlation coefficient R^2 was found to be 0.994. The proposed method was also validated for system suitability, specificity. %RSD of the drug was found to be 0.22%. After performing the accuracy studies the percentage recovery of lenvatinib was 100.8%.
Different stability studies like peroxide degradation, Photolytic degradation, Acid degradation, Alkaline degradation, Thermal degradation was also performed and result obtained was less than 1% which is under acceptance value.
From results the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Lenvatinib Educational institutions and Quality control laboratories.

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