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A SIMPLE METHOD DEVELOPMENT, VALIDATION AND QUANTIFICATION OF LENVATINIB IN PHARMACEUTICAL DOSAGE FORM USING ZERO ORDER AND FIRST-ORDER DERIVATIVE NOVEL SPECTROPHOTOMETRIC METHODS

Mona R. Patel [*1] and Harsha U. Patel [2]

Gujarat Technological University [1], Chandkheda, Ahmedabad - 382424, Gujarat, India.
Department of Quality assurance and Pharmaceutical Chemistry [2], Faculty of Pharmacy, SSSRGI, Vadasma, Mehsana - 382705, Gujarat, India.

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Correspondence to Author:
Ms. Mona R. Patel

Research Scholar,
Gujarat Technological University,
Chandkheda, Ahmedabad - 382424,
Gujarat, India.

E-mail: patelmona4795@gmail.com

ABSTRACT: Simple, very Sensitive, rapid, and accurate novel spectrophotometric methods were developed to estimate Lenvatinib in Pharmaceutical dosage form using zero- and first-order derivative spectrophotometry. The solutions of Lenvatinib standard and sample were prepared using analytical grade methanol. The spectrophotometric estimation of lenvatinib was carried out at 240 nm in the zero-order method and 253 nm in the first-order derivative method. Developed methods were validated with respect to linearity, precision, accuracy, limit of detection and quantitation in accordance with International Council for Harmonisation (ICH) guidelines. Linearity was observed in the concentration range of 1-5 µg/mL for zero- and first-order derivative methods. LOD and LOQ of Lenvatinib were found to be 0.017 µg/mL and 0.052 µg/mL for zero-order and 0.165 µg/mL and 0.500 µg/mL for first-order derivative spectrophotometric methods, respectively. The %RSD values for inter-day and intra-day precision study were <2.0%. The method was linear, accurate and precise with recoveries in the range of 98 – 102 %, and minimum values of %RSD indicate the method's accuracy. Developed spectrophotometric methods were successfully applied to assay Lenvatinib Capsule dosage form. The detailed quantitative results showed that this method is accurate, precise, and cost-effective and can be used for routine analysis of Lenvatinib in the capsule dosage form.

INTRODUCTION: Lenvatinib belongs to Anticancer Drug, a tyrosine kinase inhibitor [1]. IUPAC name, chemical formula, and Mol. Wt. of Lenvatinib are 4-[3-Chloro-4-(cyclopropyl-carbamoylamino) phenoxy]-7-methoxy-quinoline-6-carboxamide [1], $C_{21}H_{19}ClN_4O_4$ and 426.86 g/mol respectively. It is not official in any pharmacopeia [1-2].

It is freely soluble in methanol and sparingly soluble in acetonitrile and water. It is an oral anticancer drug mainly used for thyroid cancer treatment. The chemical structure of Lenvatinib is shown in **Fig. 1.**

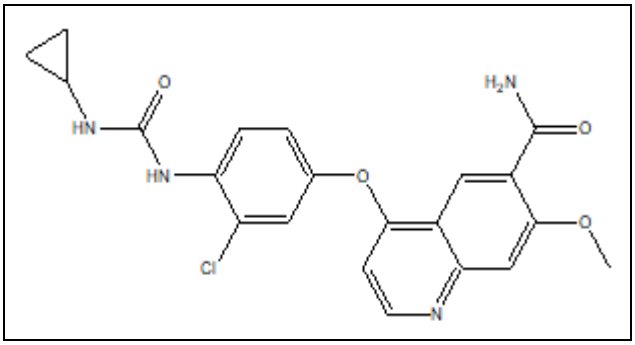

FIG. 1: CHEMICAL STRUCTURE OF LENVATINIB

QUICK RESPONSE CODE

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A detailed review of the Literature survey reveals that very few conventional methods in bulk and pharmaceutical formulation like UV Spectro-photometric methods [3], RP- HPLC [3-7], UPLC [8-9], Bioanalytical [10-11] and LC-MS [12] were reported and found to be more time consuming and expensive. The present study aimed to develop a novel, simple, rapid, and accurate and validate zero-order and first-order derivative spectrophotometric method for estimating Lenvatinib in Capsule dosage form.

EXPERIMENTAL WORK:

Instrumentation: The various instrument(s) and apparatus used during the research work are listed in **Table 1.**

TABLE 1: LIST OF INSTRUMENT(S) AND APPARATUS

Instrument(s) and Apparatus	Specifications
Double-beam UV Spectrophotometer	Shimadzu- 1800, Software-UV Probe version 2.42
Analytical balance	Reptech
Volumetric flask (borosil)	10, 50, 100 ml
Pipette (borosil)	1, 2, 5, 10 ml

Reagents and Materials: All reagents/ chemicals used during research work were either AR. The various Reagents and Chemicals used during the experiment are listed in **Table 2.**

TABLE 2: LIST OF REAGENTS AND MATERIALS

Reagents and Materials	Grade
Lenvatinib procured from Shashi Pharma as a gift sample	Reference
Lenvatinib Capsule was procured from Local Pharmacy store	---
Methanol	Analytical

Preparation of Standard Stock Solution: Weigh and transfer 0.5 mg of Lenvatinib powder in 50 mL volumetric flask containing 40 mL methanol, Shake well. Later dilute to volume with methanol to obtain the strength concentration, i.e. 10 µg/mL (Stock solution).

Preparation of Working Standard Solution: Take 5 mL from the Lenvatinib stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by methanol to obtain 5 µg/mL.

Selection of Detection Wavelength: To determine optimum λmax, Lenvatinib 5 µg/mL of working standard solution was prepared and scanned in UV visible range of 400 nm – 200 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 240 nm and 253 nm for zero order and first-order derivative spectroscopy, respectively, which was chosen for detection wavelength for estimation of Lenvatinib.

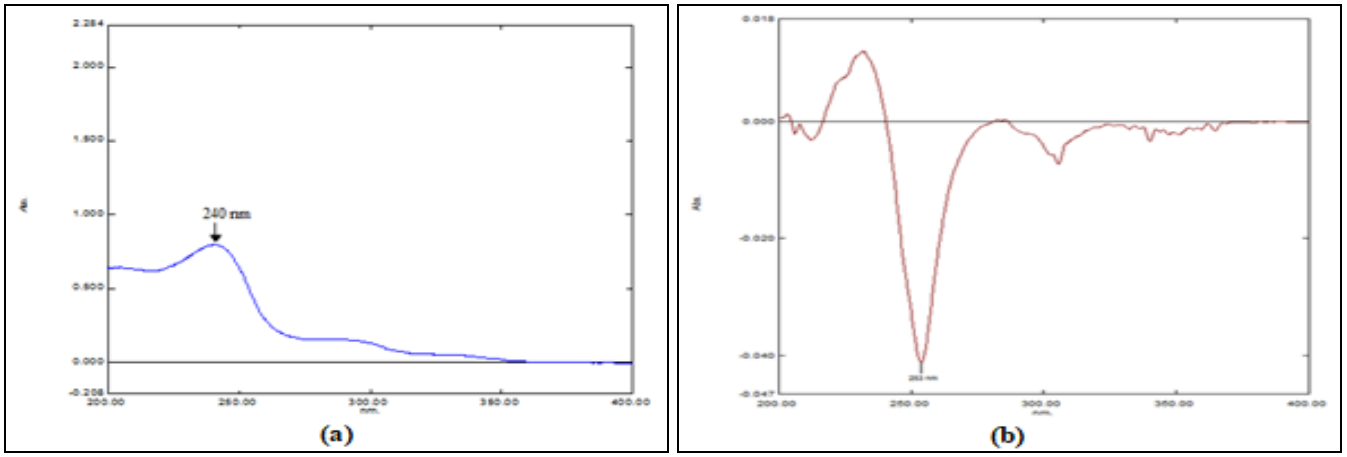

FIG. 2: (A) ZERO-ORDER SPECTRA OF LENVATINIB (5 MG/ML) IN METHANOL; (B) FIRST-ORDER SPECTRA OF LENVATINIB (5 MG/ML) IN METHANOL

Spectrophotometric Estimation of Lenvatinib by Zero Order and First Order Derivative Methods:

Linearity and Range: A calibration curve was plotted over a concentration range of 1-5 µg/mL for Lenvatinib. Precisely measured standard solution of Lenvatinib (1, 2, 3, 4 and 5 mL) was shifted to a series of 10 mL volumetric flasks and the volume was filled up to 10 mL with methanol. The calibration curve was done by plotting lenvatinib concentration on the X-axis and their respective absorbance was on the Y- axis.

System Precision:

Repeatability: In system precision, 3 µg/mL concentrations of 6 measurements of absorbance at 240 nm for zero-order and 253 nm for first order derivative were observed on the same day and responses were evaluated. The Mean, SD and %RSD were calculated.

Intermediate Precision: The Precision of developed analytical methods demonstrated by Intraday and Interday Precision.

Intraday Precision: In Intraday Precision, Standard solution containing Lenvatinib (3 µg/mL) was measured at 240 nm for zero-order and 253 nm for first-order derivative on the same day (0 hr, 3 hr and 6 hr) and then average absorbance and % RSD was calculated.

Interday Precision: In Interday Precision, the Standard solution containing Lenvatinib (3 µg/mL) was measured at 240 nm for zero-order and 253 nm for first-order derivative in three different days (day 1, 2 and 3) and then average absorbance and % RSD was calculated.

Accuracy (Recovery Studies): A recovery study of Lenvatinib was carried out by utilizing the standard addition method. By preparing the known amount of standard Lenvatinib drug at 3 levels (80%, 100%, and 120%) was added to the pre-analyzed sample and again re-analyzed. From the amount of Lenvatinib found, % recovery was estimated. The %RSD was calculated.

Limit of Detection: The LOD of an analytical method is the lowest amount of analyte in a sample that can be detected but not necessarily quantified.

Limit of Quantitation: The LOQ of an analytical method is the lowest amount of analyte in a sample which can be quantified but not necessarily detected.

Robustness: The solution was checked in UV spectrophotometer by changing wavelength; three samples were checked at 239 nm, 240 nm and 241 nm for zero-order and at 252 nm, 253 nm and 254 nm for first-order derivative methods. The mean absorbance, SD, and %RSD were calculated.

Assay: Twenty Lenvatinib Capsules were accurately weighed, and the average weight was calculated. Weigh a quantity of mixed content of above capsules containing about 4 mg of Lenvatinib was transferred to 100 ml volumetric flask. The addition of 80 ml methanol extracted Lenvatinib.

The above solution was sonicated for 20 min. and then filtered into 100 ml volumetric flask and fill up to mark with same solvent. Further dilution was made to obtain 4 µg/mL with methanol for zero-order and first-order derivative methods. Then the solution was analyzed using the proposed spectrophotometric method.

RESULT AND DISCUSSION:

Linearity and Range: Linearity was assessed by analysis of standard solution in a range of 1-5 µg/mL Lenvatinib. For zero order, the Calibration curve for Lenvatinib was found to be y= 0.152x + 0.023. The correlation coefficient (r^2) was found to be 0.991.

For first-order derivative, the Calibration curve for Lenvatinib was found to be y= 0.008x + 0.003. The correlation coefficient (r^2) was found to be 0.994. The linearity data and calibration curve results are shown in **Table 3** and **Fig. 3**, **Fig. 4,** and **Fig. 5**.

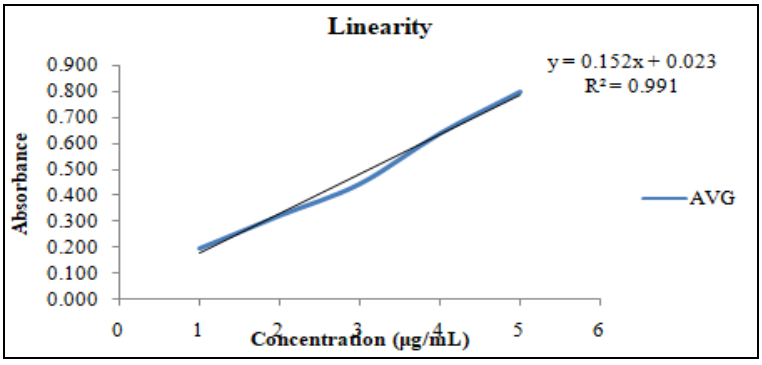

FIG. 3: STANDARD CALIBRATION CURVE OF LENVATINIB AT 240 NM (1-5 MG/ML)

TABLE 3: LINEARITY DATA FOR LENVATINIB AT 240 NM AND 253 NM (1-5 µg/mL)

Sr. no.	Conc. (µg/mL)	Zero order (240 nm)		First order derivative (253 nm)	
		Abs. ± SD	% RSD	Abs. ± SD	% RSD
1	1	0.196 ± 0.0037	1.903	0.011 ± 0.0002	1.869
2	2	0.322 ± 0.0029	0.887	0.018 ± 0.0003	1.828
3	3	0.444 ± 0.0023	0.506	0.024 ± 0.0004	1.689
4	4	0.639 ± 0.0014	0.215	0.034 ± 0.0005	1.504
5	5	0.798 ± 0.0017	0.215	0.041 ± 0.0005	1.269

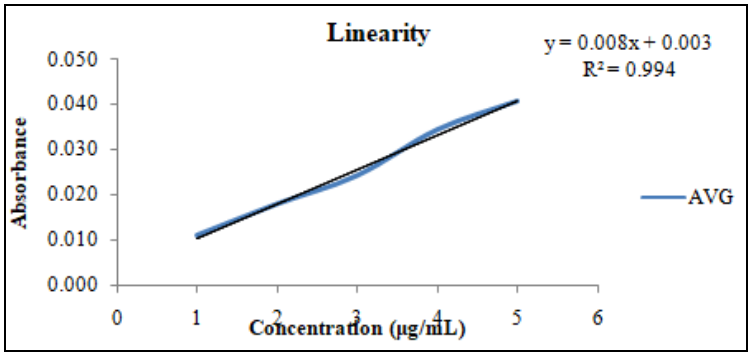

FIG. 4: STANDARD CALIBRATION CURVE OF LENVATINIB AT 253 NM (1-5 MG/ML)

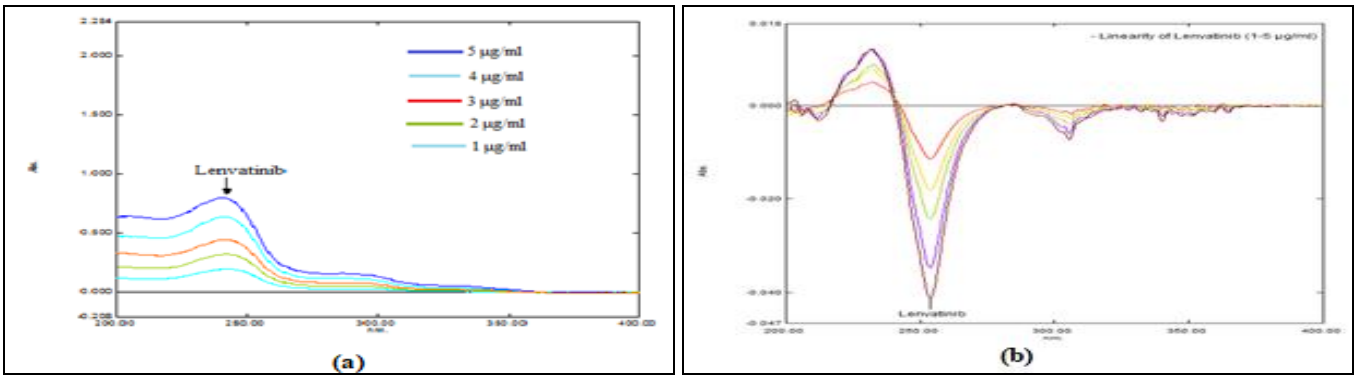

FIG. 5: OVERLAY SPECTRA OF (a) LINEARITY FOR LENVATINIB AT 240 nm; (b) LINEARITY FOR LENVATINIB AT 253 nm

System Precision (Repeatability): Repeatability study carried out using Lenvatinib solution containing 3 µg/mL. Then % RSD was found to be 0.485 for zero-order and 1.746 for first order derivative methods.

TABLE 4: REPEATABILITY DATA FOR LENVATINIB AT 240 nm FOR ZERO ORDER METHOD

Sr. no.	Conc. (µg/mL)	Repeatability		
		Absorbance at 240 nm	Mean ± SD	%RSD
1.	3	0.444	0.440 ± 0.0021	0.485
		0.448		
		0.442		
		0.445		
		0.446		
		0.443		

TABLE 5: REPEATABILITY DATA FOR LENVATINIB AT 253 nm FOR FIRST ORDER DERIVATIVE METHOD

Sr. no.	Conc. (µg/mL)	Repeatability		
		Absorbance at 253 nm	Mean ± SD	%RSD
1.	3	0.024	0.024 ± 0.0004	1.746
		0.025		
		0.024		
		0.024		
		0.025		
		0.024		

Intermediate Precision:

Intraday Precision: In Intraday Precision, % RSD was found to be 1.103 for zero-order and 1.441 for first-order derivative methods.

Interday Precision: In Interday Precision, % RSD was found to be 1.645 for zero-order and 1.766 for first-order derivative methods.

TABLE 6: PRECISION DATA FOR LENVATINIB AT 240 nm AND 253 nm

Sr. no.	Spectrophotometric methods	Lenvatinib Conc. (µg/mL)	Intraday	Interday
			Absorbance (Mean, n= 6) ± SD, %RSD	Absorbance (Mean, n= 6) ± SD, %RSD
1.	Zero order method	3	0.453 ± 0.0050, 1.103	0.451 ± 0.0065, 1.441
2.	First-order derivative method		0.024 ± 0.0004, 1.645	0.025 ± 0.0004, 1.766

Accuracy (Recovery Studies): The amount of Lenvatinib was calculated, and % recovery was found satisfactory.

TABLE 7: RECOVERY DATA FOR LENVATINIB

Sr. no.	Conc. Level	Amt. taken (µg/mL)	Amt. added (µg/mL)	Total amount (µg/mL)	Amt. Recovered (µg/mL)	% Amt. Found ± SD (mg/mL)	% RSD
Zero-order Spectrophotometric method							
1.	80%	3	2.4	5.4	2.430	101.26 ± 0.4300	0.425
2.	100%	3	3.0	6.0	2.986	99.55 ± 0.2252	0.226
3.	120%	3	3.6	6.6	3.623	100.66 ± 0.3907	0.388
First-order Derivative Spectrophotometric method							
1.	80%	3	2.4	5.4	2.409	100.38 ± 1.0752	1.071
2.	100%	3	3.0	6.0	2.996	99.88 ± 0.8438	0.845
3.	120%	3	3.6	6.6	3.616	99.40 ± 0.3976	0.400

Limit of Detection and Quantitation: The LOD was found to be 0.017 µg/mL for zero-order and 0.165 µg/mL for first-order derivative methods. The LOQ are found to be 0.052 µg/mL for zero-order and 0.5 µg/mL for first-order derivative methods.

TABLE 8: LOD AND LOQ DATA FOR LENVATINIB

Zero-order Spectrophotometric method	
LOD = 3.3 X (SD/ Slope)	**LOQ = 10 X (SD/ Slope)**
= 3.3 X (0.0008 / 0.152)	= 10 X (0.0008 / 0.125)
= 0.017 µg/mL	= 0.052 µg/mL
First-order derivative Spectrophotometric method	
LOD = 3.3 X (SD/ Slope)	**LOQ = 10 X (SD/ Slope)**
= 3.3 X (0.0004 / 0.008)	= 10 X (0.0004 / 0.008)
= 0.165 µg/mL	= 0.5 µg/mL

Robustness: The robustness of the method is demonstrated in **Table 9:**

TABLE 9: ROBUSTNESS DATA FOR LENVATINIB

Zero-order Spectrophotometric method				
Sr. no.	**Concentration**	**Abs. at 239 nm**	**Abs. at 240 nm**	**Abs. at 241 nm**
1.	3 µg/mL	0.422	0.441	0.432
2.		0.418	0.449	0.426
3.		0.432	0.438	0.419
Mean		0.424	0.443	0.426
SD		0.007	0.006	0.007
% RSD		1.700	1.284	1.529
First-order derivative Spectrophotometric method				
Sr. no.	**Concentration**	**Abs. at 252 nm**	**Abs. at 253 nm**	**Abs. at 254 nm**
1.	3 µg/mL	0.025	0.023	0.023
2.		0.025	0.024	0.024
3.		0.024	0.024	0.023

Mean	0.025	0.024	0.023
SD	0.0004	0.0004	0.0003
% RSD	1.811	1.489	1.287

Assay: The assay of the Lenvatinib Capsule dosage form was found within the standard range.

TABLE 10: ASSAY OF LENVATINIB CAPSULES

Sr. no.	Label claim (mg)	Mean Absorbance* of Sample	Mean Result* (mg)	Average* % Assay	SD	% RSD
Zero-order Spectrophotometric method						
1.	4.0	0.638	3.998	99.96	0.060	0.060
First-order derivative Spectrophotometric method						
1.	4.0	0.035	3.988	99.71	0.699	0.701

* Average of five determinations (n=5).

TABLE 11: SUMMARIZED VALIDATION PARAMETERS OF LENVATINIB

Parameters	Zero-order spectrophotometric method	First-order derivative spectrophotometric method
Concentration Range	1-5 µg/mL	1-5 µg/mL
Regression equation	y = 0.152x + 0.023	y = 0.008x + 0.003
Regression coefficient (R^2)	0.991	0.994
LOD	0.017 µg/mL	0.165 µg/mL
LOQ	0.052 µg/mL	0.5 µg/mL
Repeatability (% RSD)	0.485	1.746
Intraday precision (n=3) (Mean ± SD, % RSD)	0.453 ± 0.0050, 1.103	0.024 ± 0.0004, 1.645
Interday precision (n=3) (Mean ± SD, % RSD)	0.451 ± 0.0065, 1.441	0.025 ± 0.0004, 1.766
% Recovery	80 % 101.26 ± 0.4300, 0.425	100.38 ± 1.0752, 1.071
	100 % 99.55 ± 0.2252, 0.226	99.88 ± 0.8438, 0.845
	120 % 100.66 ± 0.3907, 0.388	99.40 ± 0.3976, 0.400
Assay of Marketed formulation	99.96 %	99.71 %

CONCLUSIONS: No derivative spectrophotometric methods have been described to estimate Lenvatinib in the capsule dosage form. Therefore very simple, novel, fast, sensitive, and accurate, as well as validated zero-order and first-order derivative spectrophotometric methods, were developed and validated according to the ICH guidelines. This method is linear over the 1- 5 µg/mL concentration range. The system's suitability, precision, and accuracy values are within acceptable limits. Developed spectro-photometric methods were successfully applied to assay Lenvatinib in the capsule dosage form. The detailed quantitative results showed that this method is accurate, precise, and cost-effective and can be used for routine estimation of Lenvatinib in the capsule dosage form.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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