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Research Article

**METHOD DEVELOPMENT AND VALIDATION OF
LENVATINIB DRUG BY RP-HPLC IN PHARMACEUTICAL
DRUG DOSAGE FORM****Y. Prashanthi¹, Mohammed Asif Ahmed², K. Vijaya³, Riyazuddin^{*4}**¹Dept. of Chemistry, Mahatma Gandhi University, Nalgonda, Telangana-508254, India²Dept. of Food Science & Nutrition, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia³Dept. of Pharmaceutical Chemistry, TKR college of Pharmacy, Hyderabad, Telangana-5000097, India⁴Dept. of Food Science & Nutrition, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia**Abstract:**

A novel approach has used for the development and validates a rapid, simple and an isocratic RP-HPLC method with PDA detector for the estimation of Lenvatinib drug in pharmaceutical drug dosage form. Lenvatinib was separated using YMC C 18, 4.6 X 150 mm, 5 μ analytical column, a Waters HPLC system and a mobile phase consists of water and methanol in the ratio of 30:70 % v/v, delivered at 0.6 ml/min with 20 μ l injection volume. The detector wavelength was at 240 nm. The system suitability parameters for Lenvatinib drug such as theoretical plates and tailing factor were found to be 4384.4, 1.6 respectively. Linearity was established for Lenvatinib in the range of 20 – 100 μ g/ml concentration levels with a correlation coefficient of (r^2) 0.999. The repeatability and reproducibility precision values were found to be 0.75 and 0.31 % respectively. The accuracy values were found to be in the range of 98 – 102 %. The limits of detection and quantification values were 0.48 μ g/ml and 1.46 μ g/ml respectively. The method was validated for specificity, system suitability, precision, accuracy, LOD and LOQ parameters as per the ICH guidelines. This method can be used for the estimation and analysis of Lenvatinib drug in active pharmaceutical ingredients and pharmaceuticals.

Keywords: Lenvatinib, Lenvima, High Performance Liquid Chromatography, Photo Diode Array detector, Estimation

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INTRODUCTION:

Thyroid cancer is the increasing incidence in the United States. Average annual percentage increases from 2006 to 2010 for thyroid cancer were 4.5 % for men and 6.5 % for women [1]. Thyroid cancer is a common malignant endocrine tumor, which has recently been increasing. Above 90 % of thyroid cancers are the follicular or capillary types known as differentiated thyroid cancer (DTC) [2] which comprises 1 % of all cancers globally. 90 % of DTC patients survive at least 10 years only [3]. Various genetic alterations participate in the tumorigenesis of thyroid cancer [4]. Across all thyroid cancers, 5 years relative survival rates are high about 98 %; however, about 30 % of patients has disease recurrence [1, 5]. Tumor angiogenesis inhibition by blocking with small molecular vascular endothelial growth factor (VEGFR) signaling is a promising therapy for thyroid cancer. Lenvatinib is a potent VEGF inhibitor. Lenvatinib (Trade name Lenvima) is an anticancer drug for the treatment of certain kinds of thyroid cancers. It acts as multiple kinase inhibitors against VEGF-1, VEGF-2, and VEGF-3 kinase [6]. Lenvatinib is very effective against the thyroid cancer, but it does not respond with radioactive iodine [7,8]. Lenvatinib is a prescription medication used to treat a type of thyroid cancer. Lenvatinib belongs to a group of drugs called kinase inhibitors, which works by blocking certain proteins that helps cancer cells grow and divide. The structure of Lenvatinib drug was presented in the Figure No.1

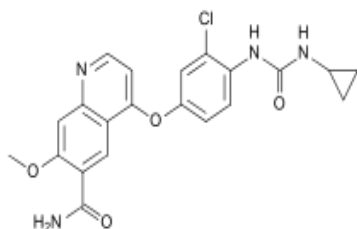


Fig. 1: Structure of Lenvatinib drug

On May 13, 2016, the U. S. Food and Drug Administration approved lenvatinib capsules (Lenvima, Eisai, Inc.), in combination with everolimus, for the treatment of advanced renal cell carcinoma following one 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 [9].

Literature review reveals that very few analytical methods have been reported for the determination of Lenvatinib drug which includes High Performance Liquid Chromatography [10], Liquid Chromatography – mass spectroscopy [11-13], and

pharmacokinetics studies [14-16]. The present study was aimed to develop a simple and accurate RP-HPLC-PDA method for the estimation of Lenvatinib drug according to ICH guidelines [17].

MATERIAL AND METHODS:**Chemicals:**

A pure compound of Lenvatinib drug was gifted by MSN Pharmaceuticals Private Limited (Hyderabad, India). Acetonitrile, Methanol and Water were of HPLC grade purchased from Merck (Germany). Ortho phosphoric acid, potassium di hydrogen phosphate and di potassium mono hydrogen phosphate were of General reagent grade purchased from Merck (Germany). 0.22 μ nylon filters HPLC grade were from advance lab (India). 0.45 μ filter papers were HPLC Grade from Millipore (USA).

Equipment:

A Waters HPLC instrument system (Waters, USA) separation module 2695, consist of 2487 UV detector and 996-PDA was used for the method development and validation studies. For the data acquisition and integration, empower software version-2 was used. An ultra-sonic water bath sonicator model SE60US, Enertech company (USA), digital PH meter (AD 102U, ADWA), digital weighing balance ER 200A from Ascocet (USA) and UV double beam spectrometer (UV 3000⁺ UV win software) (Labindia, India) were used in the study.

Chromatographic conditions:

Lenvatinib was analyzed on YMC C18 (5 μ m, 4.6x150mm) analytical column. For the elution of Lenvatinib drug we used the mobile phase consists of Water: Methanol in the ratio of 30:70% v/v, delivered at 0.6 ml/min. The mobile phase was filtered through 0.22 μ nylon filters under vacuum filtration. The injection volume was 20 μ l and the detector was set at the wavelength of 240 nm. The auto-sampler and column were maintained at ambient temperature condition. The chromatographic program runtime was 7.0 minutes.

Preparation of mobile phase:

The mobile phase used under the chromatographic conditions was prepared by the mixing of HPLC grades of water and methanol in the ratio of 30:70 % v/v. Then this mobile phase mixture was degassed by using an ultrasonic water bath for 5 minutes and filtered through 0.22 μ nylon filters under vacuum filtration system.

Preparation of diluent:

Mobile phase was used as diluent.

Preparation of sample solution of Lenvatinib drug:

10 mg of Lenvatinib tablet powder was accurately weighed and transferred into a 10 ml clean dry

volumetric flask, added about 2 ml of diluent (mobile phase) and sonicated to dissolve it completely and made volume up to the mark with diluent. Further pipette out 10 ml of the above stock solution into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of standard solution of lenvatinib drug :

10 mg of Lenvatinib working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and added 2 ml of diluent and sonicated to dissolve it completely and make up to the mark with diluent. Further pipette out 1 ml of the above solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Validation parameters:

Selection of wavelength:

Prepared 10 µg/ml concentration of Lenvatinib drug in the mobile phase and scanned by using the UV-double beam spectrometer with in the wavelength region of 200-400 nm. The maximum absorption wavelength was selected for the detection of Lenvatinib wavelength.

Percentage of assay:

10 µl solution from standard and sample solution of Lenvatinib drug were injected in triplicate into the chromatographic system and the peak areas were measured for Lenvatinib drug. The percentage of assay was calculated by comparing the peak area of standard and sample chromatogram by using formula. Formula;

$$\text{Assay \%} = \frac{\text{sample area}}{\text{Standard area}} \times \frac{\text{dilution sample}}{\text{dilution of standard}} \times \frac{P}{100} \times \frac{\text{Avg.wt}}{Lc} \times 100$$

Where,

P - percentage purity of working standard

Lc - label claim of drug in mg/ml

System suitability test:

At first the HPLC system was optimized as per the chromatographic conditions. 10 µl solution from standard and sample solution of Lenvatinib drug were injected in triplicate into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, and tailing factor were calculated.

Specificity:

The specificity of the method was carried out to check whether there is any interference of any impurities in the retention time of analytical peak. The specificity was performed by the injection of

blank, standard and sample solution of Lenvatinib drug.

Linearity:

Prepared 20, 40, 60, 80, and 100 µg/ml concentration levels of calibration standard solutions of Lenvatinib drug and injected into the chromatographic system. A linear regression was used to plot the calibration graph of peak area (on y – axis) versus concentration (on x - axis) of Lenvatinib. Each peak area was used to calculate the correlation coefficient (r^2).

Accuracy:

The accuracy of the method was determined by calculating the percentage of recovery values of Lenvatinib drug by the method of standard addition. Known amount of standard solution of Lenvatinib at 50, 100 and 150 % were added to pre-quantify the sample solution of Lenvatinib drug and injected into the chromatographic system. Each standard solution was prepared in triplicate and analyzed. The peak area at each level was used to calculate the percentage of recovery at each level.

Limit of detection and limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as $3.3 \times SD/S$ and $10 \times SD/S$ respectively as per ICH guidelines . Where SD is the standard deviation 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 3). The LOQ is the smallest concentration of analyte which gives the response that can be accurately quantified (signal to noise ratio 10).

Precision studies:

Method precision (Repeatability):

Method precision for Lenvatinib drug solution was checked by repeatability. We prepared injection samples of concentration of 100 µg / ml of Lenvatinib drug solution and injected for five times into the system.

Intermediate precision (Reproducibility):

We performed the intermediate precision test. For evaluating the intermediate precision we analyzed six injections of 100 µg / ml concentration of Lenvatinib drug on different days by different columns of same dimensions by different analysts. Each injection area was used to calculate the % RSD.

RESULTS AND DISCUSSION:

During method development for the estimation and analysis of Lenvatinib drug we used different types of analytical columns and mobile phases.

In the first approach, Xterra RP-18 4.6 X 150 mm, 5 µ analytical column was used for the separation of drug. Methanol and water in the ratio of 60:40 % v/v mobile phase was used to elution of the drug with 1 ml/min flow rate. The injection volume was 10 µl

and the detector wavelength was set at 240 nm. The program runtime was 8.0 minutes. This trial showed no good peak separation, so we performed further trial.

In the another case, Inertsil ODS 4.5 X 150 mm, 5 μ analytical column, acetonitrile and water in the ratio of 80:20% v/v mobile phase was employed. We injected 20 μ l injection volume with 1 ml/min. The detector wavelength was set at 240 nm and the program runtime was 8.0 minutes. In this trial no peak was observed, still more trials were required.

In further trial Xterra RP-18 4.6 X 150 mm, 5 μ analytical column, acetonitrile and p^H 6.8 phosphate buffer solution in 75:25 % v/v ratio were used for the analysis. 10 μ l injection volume was injected with 1 ml/min mobile phase flow rate. Auto-sampler and column temperatures were maintained at ambient condition. The program runtime was 10 minutes. In this trial Lenvatinib was eluted but there was no proper separation. Further trials were required.

In the another case, Agilent C18 4.6 X 250 mm, 5 μ analytical column, acetonitrile and p^H 3.0 phosphate buffer solution in the ratio of 60:40% v/v mobile phase was employed. We injected 10 μ l injection volume with 1 ml/min. The detector wavelength was set at 240 nm and the program runtime was 20 minutes. In this trial Lenvatinib peak was well separated with good peak shape. But the retention time of Lenvatinib was observed at 13.33 min. so we performed further trials to decrease the retention time of Lenvatinib drug.

In the case of last trial YMC C 18 4.6 X 150 mm, 5 μ analytical column, water and methanol in the ratio 30:70 % v/v mobile phase was used for the elution of drug. Flow rate was 0.6 ml/min. 20 μ l was the injection volume and the detector wavelength was set at 240 nm. Auto-sampler and column temperatures were maintained at ambient condition. The program run time was 7.0 minutes. In this trial, the separation was good, peak shape was good, and the retention time for Lenvatinib was observed at 2.135 min. So we conclude that there was no required for decrease the retention time of peak, so it is taken as final method. The spectra were extracted from the PDA detector in the chromatographic conditions.

In 2015, one study was reported who developed RP-HPLC-DAD method for the estimation and analysis of Lenvatinib drug [18]. In the case of reported study they got LOD and LOQ values were 1.2 and 3.8 μ g/ml respectively. They got the retention time was 3.733 minutes. In our study we used different analytical column and mobile phase when compare to the recent reported study. Here we find the retention time was 2.135 minutes, LOD and LOQ values are 0.48 and 1.46 μ g/ml respectively. Compare to the recent reported study on Lenvatinib drug our results are superior.

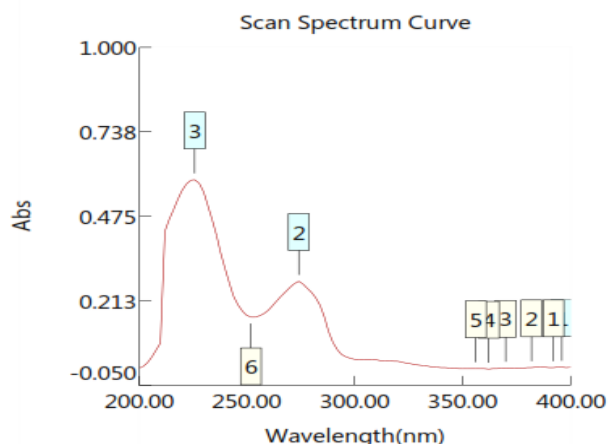


Fig. 2: UV- double spectrum of Lenvatinib drug Assay validation:

Selection of wavelength:

Lenvatinib drug was shown a maximum of UV absorbance at 240 nm. So we have taken 240 nm as a detection wavelength for Lenvatinib drug. The chromatogram was shown in Fig. No. 2.

Percentage of assay:

We performed the percentage of assay study for Lenvatinib drug and we found the percentage of purity of Lenvatinib drug in pharmaceutical drug dosage form was 99.56 %. The obtained results were shown in Table No.1.

Table 1: percentage of assay results for Lenvatinib drug

Drug	Lenvatinib capsule label claim (mg)	Amount found (mg)	% label claim \pm % RSD
Lenvatinib	10	9.95	99.56 \pm 0.24

Table 2: system suitability results of Lenvatinib drug

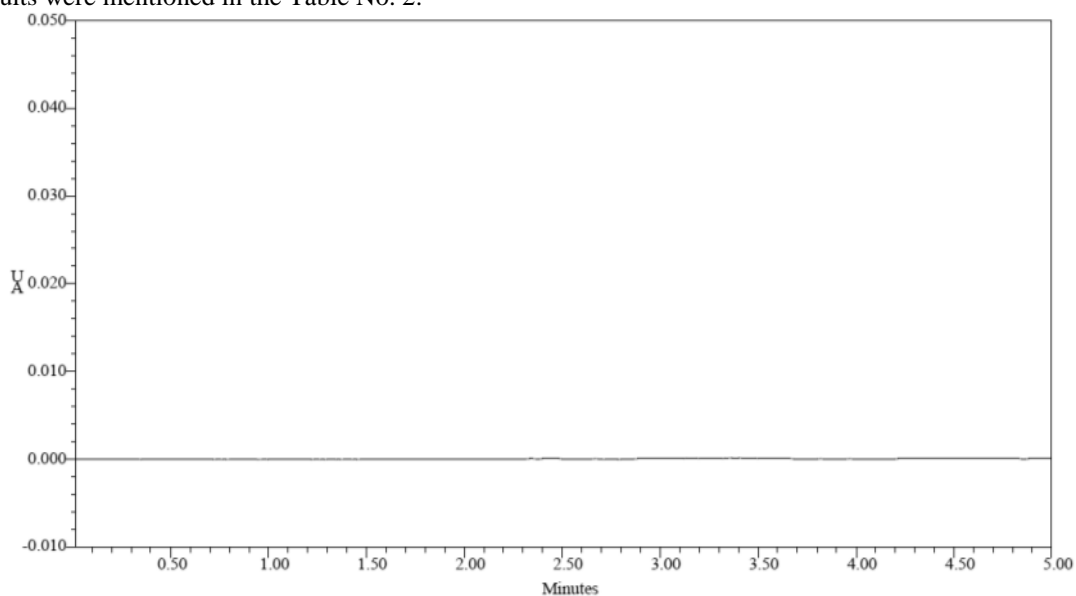
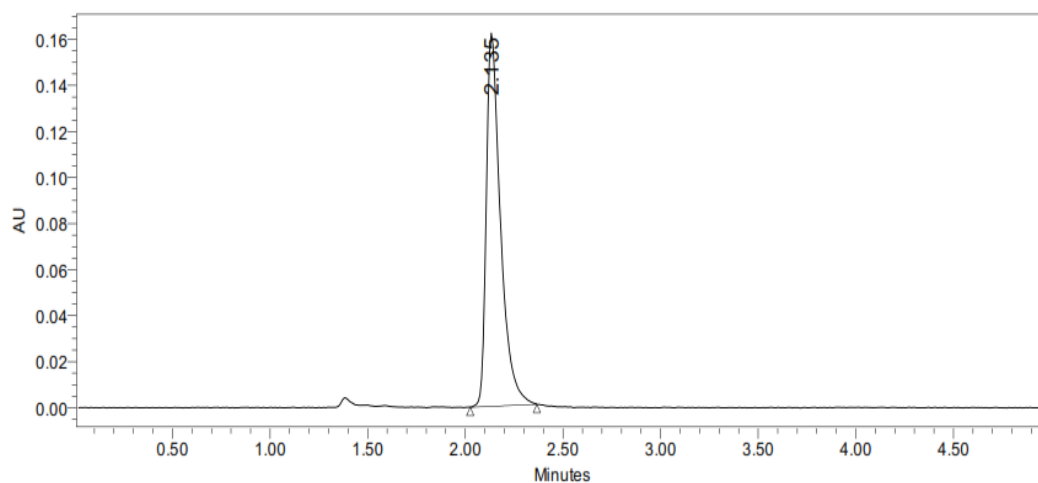
Parameter (n=6)	Lenvatinib
Retention time	2.135
Theoretical plates	4384.4
Tailing factor	1.6

System suitability:

By performing system suitability test for Lenvatinib drug, we found the retention time for Lenvatinib drug was 2.135 min. Theoretical plates and tailing factors were found to be 4384.4, 1.6 respectively. The obtained results were agreed with ICH guidelines. The results were mentioned in the Table No. 2.

Specificity:

Fig. No. 3 shows that there was no interference of impurity or any endogenous peak in retention time of Lenvatinib drug peak. The chromatograms of blank, standard and sample solution of Lenvatinib drug were shown in Fig No.3,4, and 5 respectively.

**Fig. 3: chromatogram of blank solution (Mobile phase)****Fig. 4: chromatogram of standard solution of Lenvatinib drug**

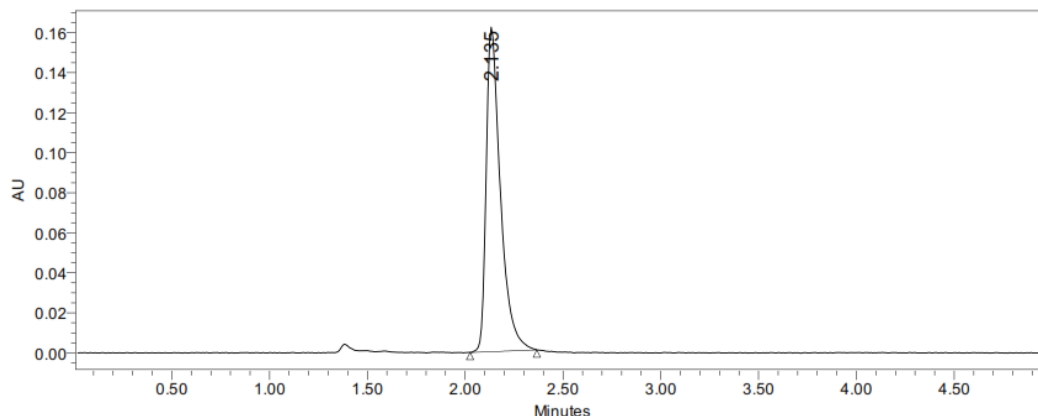


Fig. 5: chromatogram of sample solution of Lenvatinib drug

Table 3: linearity results for Lenvatinib drug

Parameters	Values ($\mu\text{g/ml}$)
Regression equation	$Y = 2604.5x - 17757$
r^2	0.999
LOQ	1.46
LOD	0.48

LOD – limit of detection, LOQ - limit of quantification

Linearity:

The linearity of the method was evaluated over a concentration range of 20 – 100 $\mu\text{g/ml}$. we found the correlation coefficient to be 0.999. a perfect linearity was obtained in the concentration versus peak area for Lenvatinib drug. The mean values for the regression equation were ($y = 2604.5x - 17757$) where y - axis was the peak area and x – axis was the concentration of Lenvatinib drug in $\mu\text{g/ml}$. The obtained results were summarized in the Table No. 3.

Accuracy:

Accuracy or recovery study was performed for 50, 100 and 150 % concentration of Lenvatinib drug. The

results were in accordance with ICH guidelines. According to ICH guidelines the percentage of recovery should be in the range of 98 – 102 %. The obtained results were summarized in the Table No. 4.

Limit of detection and limit of quantification:

LOD and LOQ were determined by analysis of standard solution of Lenvatinib drug solution. We found the LOD and LOQ were to be 0.48 and 1.46 $\mu\text{g/ml}$ respectively. The obtained values for LOD were taken on the basis of signal – to – noise ratio 3 and for LOQ is 10. These results were in agreement with ICH guidelines. The obtained results were mentioned in the Table No.5.

Table 4: Accuracy (Recovery) studies results for Lenvatinib drug

Sample name	Amount added (mg)	Amount found (mg)	% Recovery	Statistical analysis
S ₁ :50	5	4.95	99.00	Mean-99.13
S ₂ :50	5	4.96	99.20	SD-0.11
S ₃ :50	5	4.96	99.20	%RSD-0.11
S ₄ :100	10	9.86	98.60	Mean-98.73
S ₅ :100	10	9.87	98.70	SD-0.15
S ₆ :100	10	9.89	98.90	% RSD-0.15
S ₇ :150	15	14.99	99.99	Mean-99.99
S ₈ :150	15	15.00	100	SD-0.0057
S ₉ :150	15	15.00	100	% RSD-0.0057

SD – standard deviation, RSD – relative standard deviation

Precision:

Repeatability and reproducibility studies were evaluated by analyzing five Lenvatinib samples of 100 µg/ml solutions. We found the repeatability and reproducibility % RSD values were found to be 0.75

and 0.31 % respectively. These results are in agreement with ICH guidelines. According to ICH guidelines the precision value should be less than 2 %. The results of repeatability and reproducibility were mentioned in the Table No.6 and 7 respectively

Table 5: Linearity study results of Lenvatinib drug

Parameters	Values (µg/ml)
Regression equation	$Y = 2604.5x - 17757$
r^2	0.999
LOQ	1.46
LOD	0.48

LOD – limit of detection, LOQ - limit of quantification

Table 6: Repeatability (intra-day) precision data of Lenvatinib drug

Retention time	Concentration (µg/ml)	Peak Area
2.180	100	830760
2.184	100	832532
2.185	100	823385
2.188	100	840724
2.188	100	829385
		831357.4
SD		6263.20
% RSD		0.75

SD – standard deviation, RSD – relative standard deviation

Table 7: Reproducibility (intra-day) precision study data of Lenvatinib drug

Reproducibility	% RSD
Instrument 1 (n =6)	
Pa ratio (n =6)	0.31
Rt ratio (n =6)	0.12
Instrument 2 (n =6)	
Pa ratio (n =6)	0.31
Rt ratio (n =6)	0.14

Pa - peak area, Rt – retention time, n – number of determination

CONCLUSION:

A fast, accurate, and a simple an isocratic RP-HPLC method with PDA detector for the estimation and analysis of Lenvatinib drug in the pharmaceuticals was developed and validated as per ICH guidelines. Linearity was achieved for Lenvatinib drug in the range of 20 - 100 µg/ml with correlation coefficient (r^2) of 0.999. The percentage of recovery was achieved in the range of 98 – 102 % which is in the acceptance criteria of ICH guidelines. The % RSD were less than 2% which proved the precision of the developed method. The system suitability parameters were investigated to obtain the optimum chromatographic conditions. The advantage of this

method is suitable retention time and high sensitivity. The obtained results suggest that this method can be used for the routine analysis of Lenvatinib drug in the pharmaceuticals.

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