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Research Article

A new analytical method development and validation for the estimation of lenvatinib by using RP-HPLC method

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ABSTRACT

A simple and selective LC method is described for the determination of Lenvatinib dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Phosphate buffer (KH2PO4): Acetonitrile (80:20) with detection of 240nm. Linearity was observed in the range 60-140 µg /ml for Lenvatinib (r^2 =0.996) for the amount of drug estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Lenvatinib**,** Reverse phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1**].**

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis [3-5].

Qualitative analysis is performed to establish composition of a substance [6-8]. It is done to determine the presence of a compound or substance in a given sample or not. The various

qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc [9, 10].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of Lenvatinib in pharmaceutical dosage form.

PLAN OF WORK

- Solubility determination of Lenvatinib various solvents and buffers.
- Determine the absorption maxima of the drug in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines

METHODOLOGY

Mobile phase

A mixture of 80 volumes of Phosphate bufferpH4.0 and 20volumes of Acetonitrile was prepared. The mobile phase was sonicated for 10min to remove gases.

Determination of working wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of LENVATINIB

100mg of LENVITINIB was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 100 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS AND DISCUSSIONS

Solubility Studies

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

Lenvatinib

Freely soluble in methanol,water and phosphate buffer.

Wavelength determination

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of LENVATINIB

100 mg of LENVATINIB was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol, wavelength is found to be 240nm.

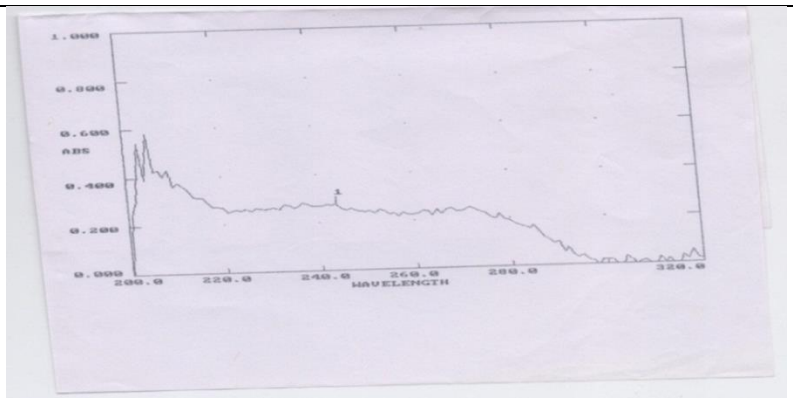


Fig: UV-VIS spectrum of LENVATINIB

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra and the absorption curve shows the isobestic point was found to be 240 nm for the combination.

METHOD DEVELOPMENT OF LENVATINIB

Trial- 1

Preparation of standard solution

Weigh accurately 100 mg of LENVITINIB in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 100 µg/ml of LENVITINIB is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

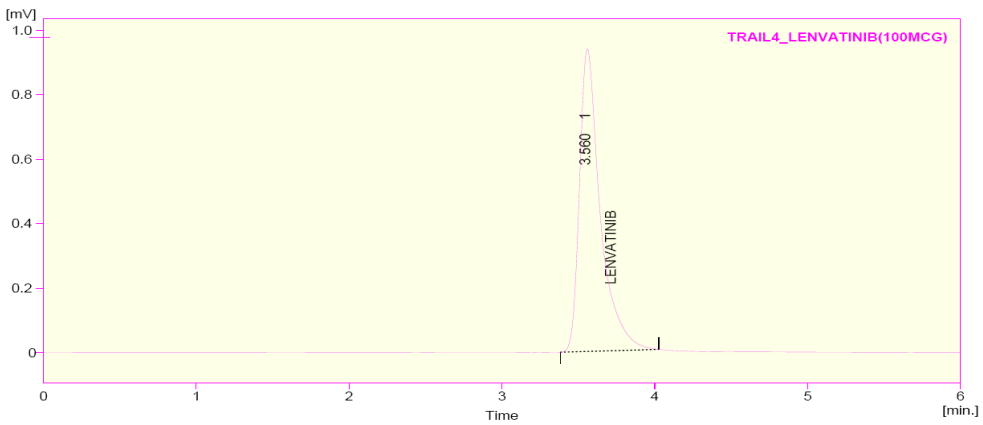


Fig: Chromatogram of LENVATINIB

Observation

- Peak Asymmetry factor for LENVATINIB meet the system suitability requirements.
- The run time is very correct.
- Theoretical plates were more than 2000. Hence it is taken for optimization.

Table 1: Optimized chromatographic conditions

Mobile phase	Phosphate buffer(KH2PO4): Acetonitrile(80:20)
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	240nm
Injection volume	20 µl
Run time	6 min
Retention time	About
Mobile phase	Phosphate buffer(KH2PO4): Acetonitrile(80:20)

Assay

Preparation of samples for Assay

Preparation of standard solution

Weigh accurately 100 mg of LENVITINIB in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 100 µg/ml of LENVITINIB is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

20 tablets (each tablet contains 62.5mg of LENVITINIB was weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of LENVITINIB (100µg/ml) were prepared by dissolving weight equivalent to 62.5 mg of LENVITINIB and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of LENVITINIB was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of LENVITINIB present in the formulation by using the formula given below, and results shown in above table

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of LENVITINIB in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

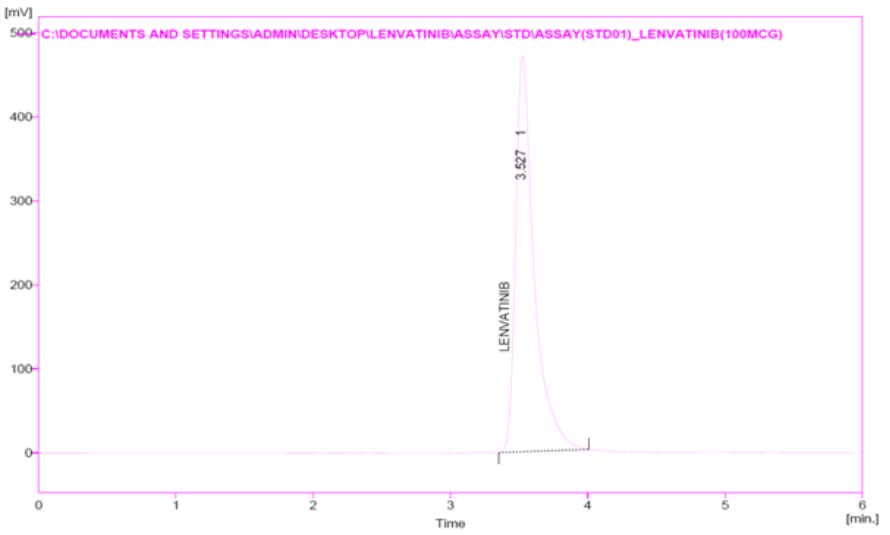


Fig: Chromatogram of Assay standard preparation-1

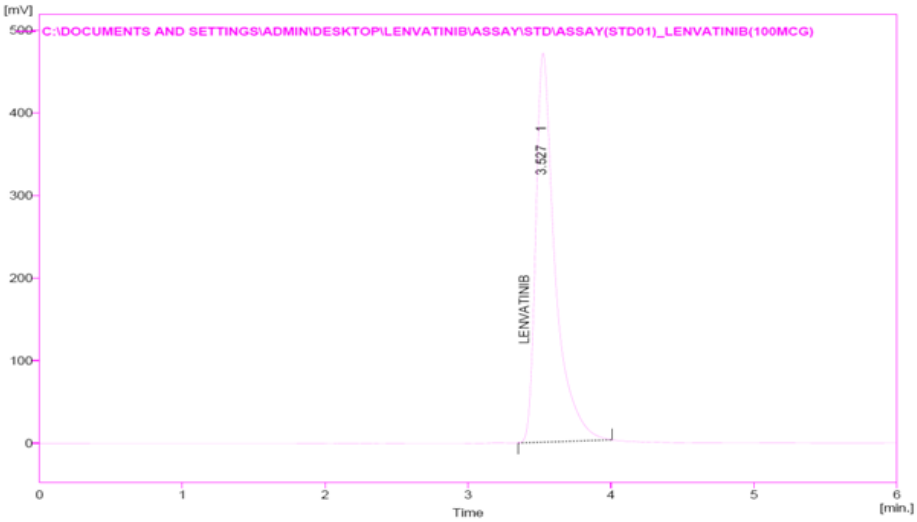


Fig: Chromatogram of Assay standard preparation-2

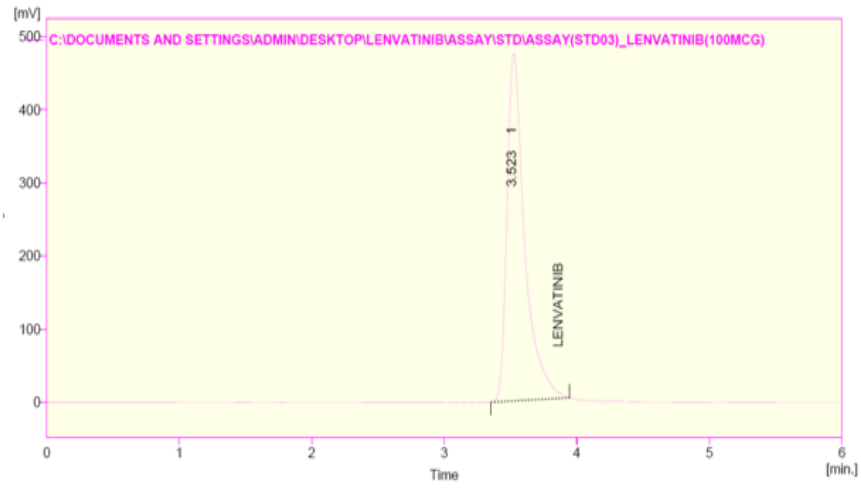


Fig: Chromatogram of Assay standard preparation-3

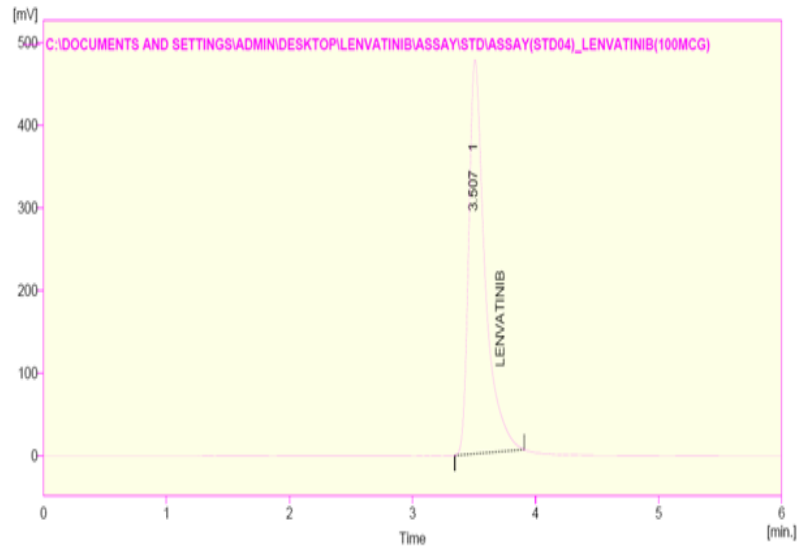
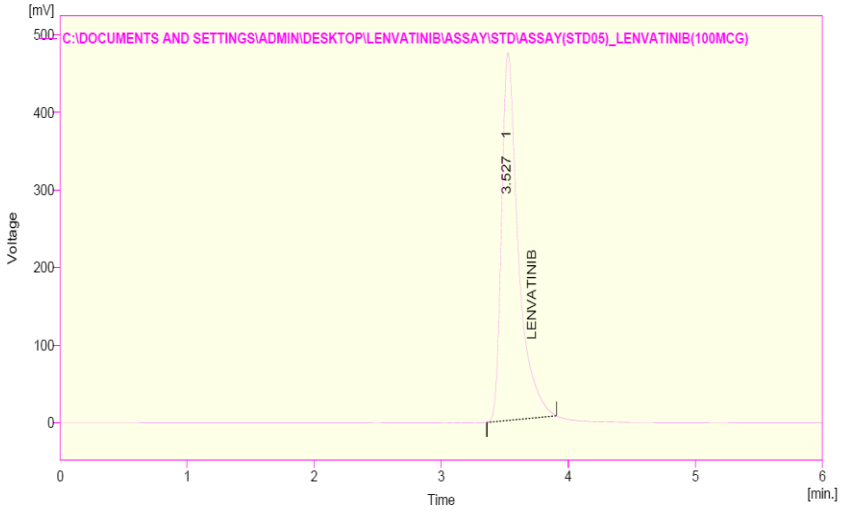


Fig: Chromatogram of Assay standard preparation-4

Sample Info:

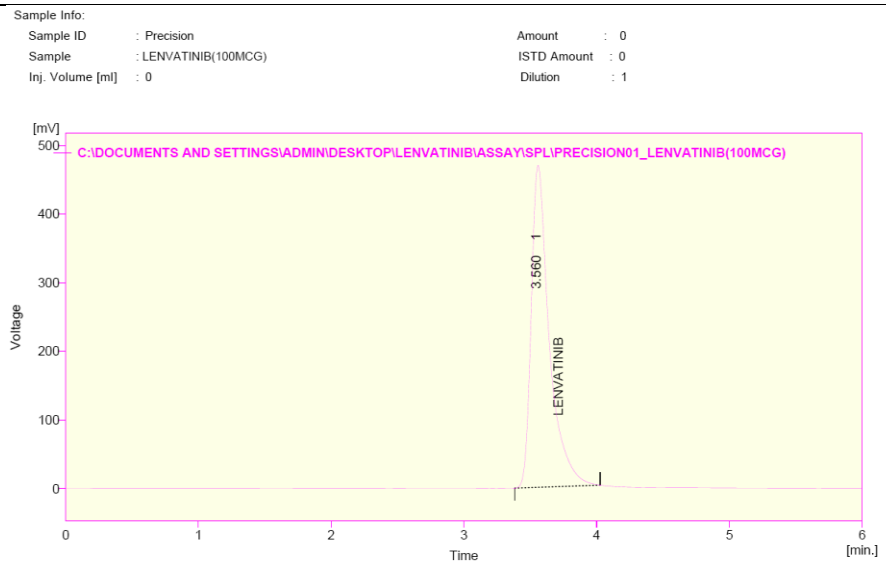
Sample ID	: Assay	Amount	: 0
Sample	: LENVATINIB(100mcg)	ISTD Amount	: 0
Inj. Volume [ml]	: 0	Dilution	: 1



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\LENVATINIB\ASSAY\STD\ASSAY(STD05)_LENVATINIB(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.527	4347.941	473.751	100.0
	Total	4347.941	473.751	100.0

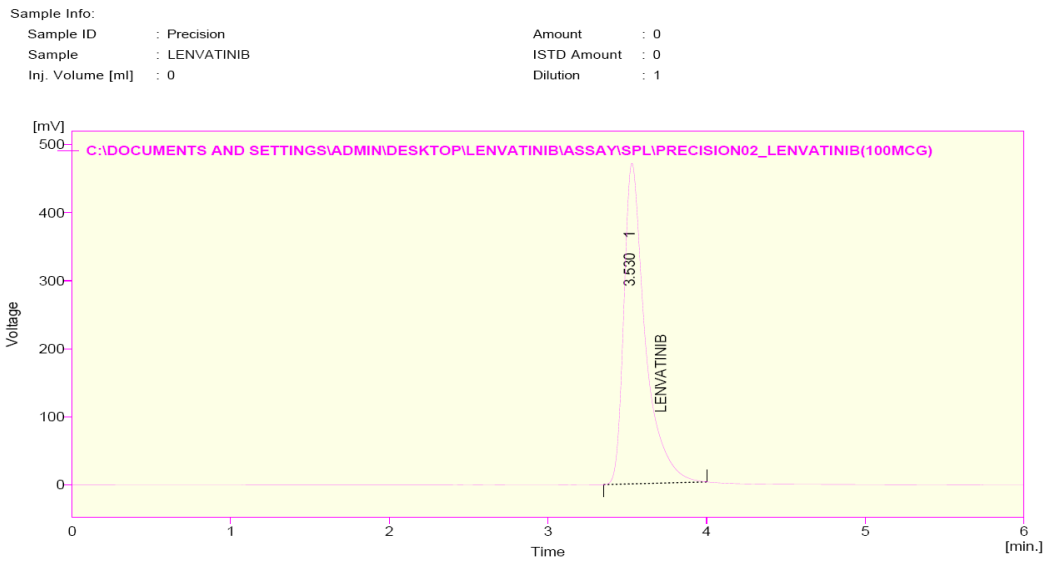
Fig: Chromatogram of Assay standard preparation-5



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\LENVATINIB\ASSAY\SPL\PRECISION 01_LENVATINIB(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.560	4426.890	469.053	100.0
	Total	4426.890	469.053	100.0

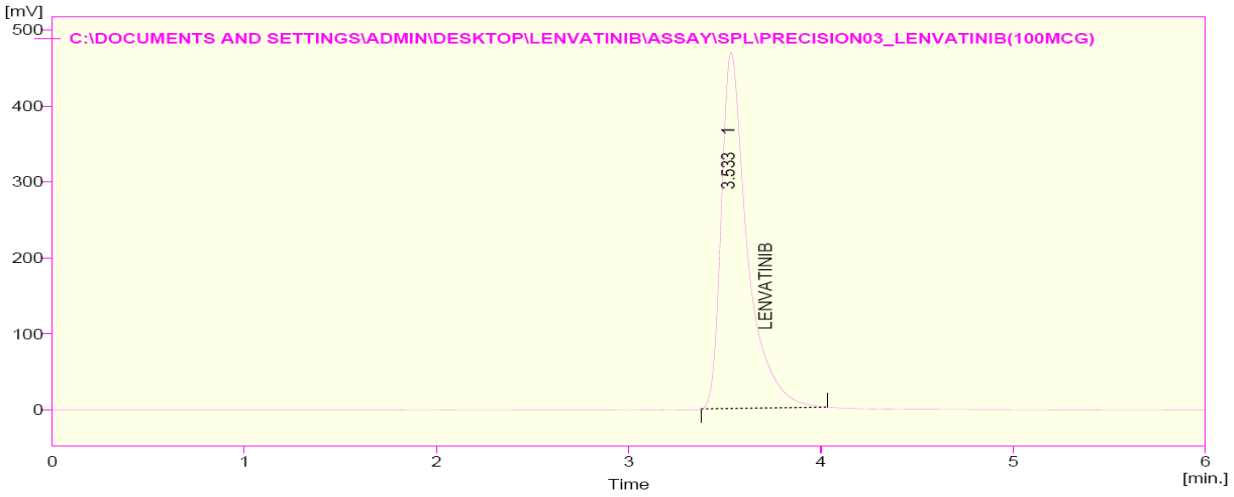
Fig: Chromatogram of Assay sample preparation-1



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\LENVATINIB\ASSAY\SPL\PRECISION 02_LENVATINIB(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.530	4417.702	471.055	100.0
	Total	4417.702	471.055	100.0

Fig: Chromatogram of Assay sample preparation-2



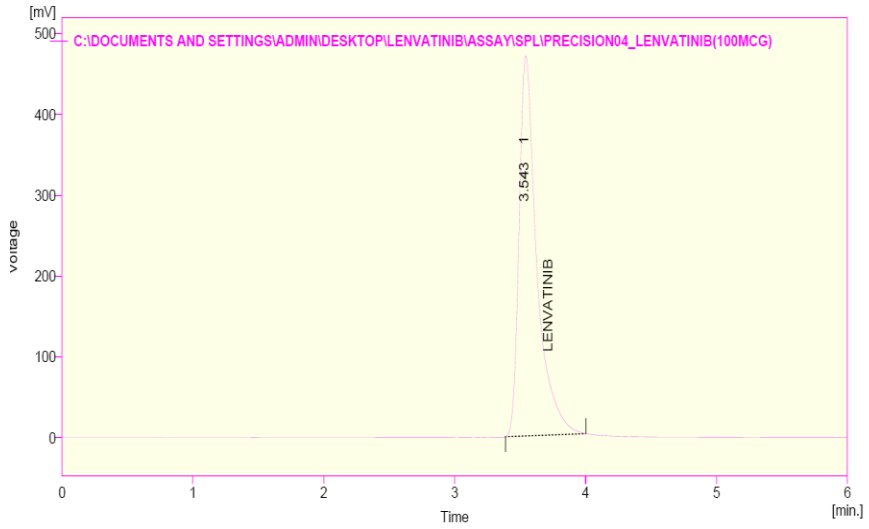
Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\LENVATINIB\ASSAY\SPL\PRECISION 03_LENVATINIB100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.533	4410.326	468.687	100.0
	Total	4410.326	468.687	100.0

Fig: Chromatogram of Assay sample preparation-3

Sample Info:

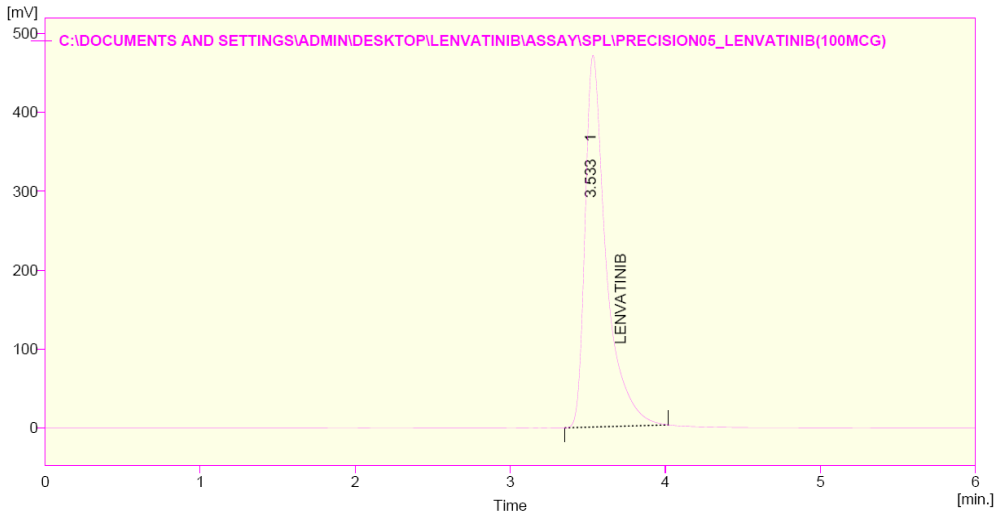
Sample ID	: Precision	Amount	: 0
Sample	: LENVATINIB	ISTD Amount	: 0
Inj. Volume [ml]	: 0	Dilution	: 1



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\LENVATINIB\ASSAY\SPL\PRECISION 04_LENVATINIB(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.543	4411.818	470.444	100.0
	Total	4411.818	470.444	100.0

Fig: Chromatogram of Assay sample preparation-4



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\LENVATINIB\ASSAY\SPL\PRECISION 05_LENVATINIB(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	3.533	4442.974	471.343	100.0
	Total	4442.974	471.343	100.0

Fig: Chromatogram of Assay sample preparation-5

Table: Assay Results

LENVITINIB		
	Standard Area	**Sample Area**
Injection-1	4430.026	4426.890
Injection-2	4447.469	4417.702
Injection-3	4387.764	4410.326
Injection-4	4376.391	4411.818
Injection-5	4347.941	4442.974
Average Area	6372.840	4426.890

Observation

The amount of LENVITINIB present in the taken dosage form was found to be 90% and 110% respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 10 mg of LENVATINIB in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 100 µg/ml of LENVATINIB is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

20 tablets (each tablet contains 10mg of LENVATINIB was weighed and taken into a mortar and crushed to fine powder and uniformly

mixed. Tablet stock solutions of LENVATINIB (100µg/ml) were prepared by dissolving weight equivalent to 10 mg of LENVATINIB and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of LENVATINIB was made by adding 1 ml of stock solution to 10 ml of mobile phase.

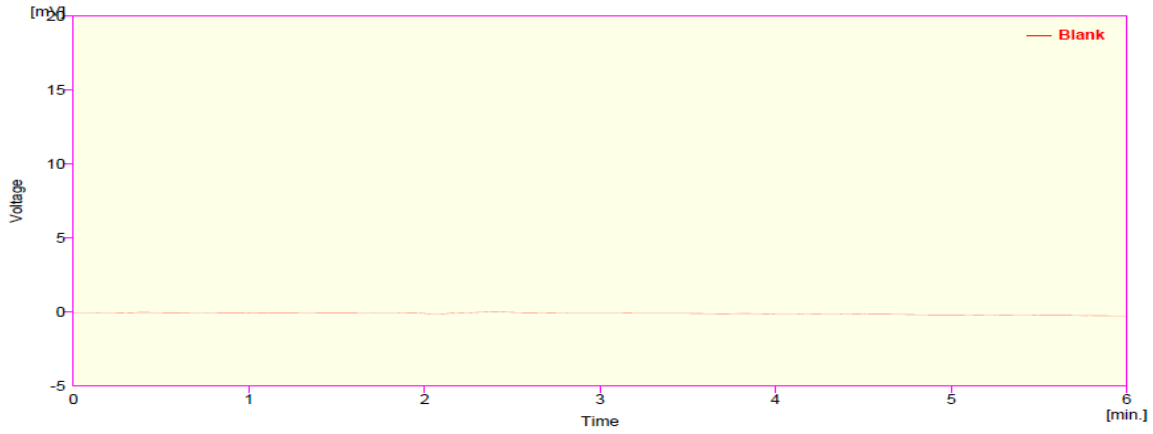


Fig: Blank chromatogram for specificity by using mobile phase

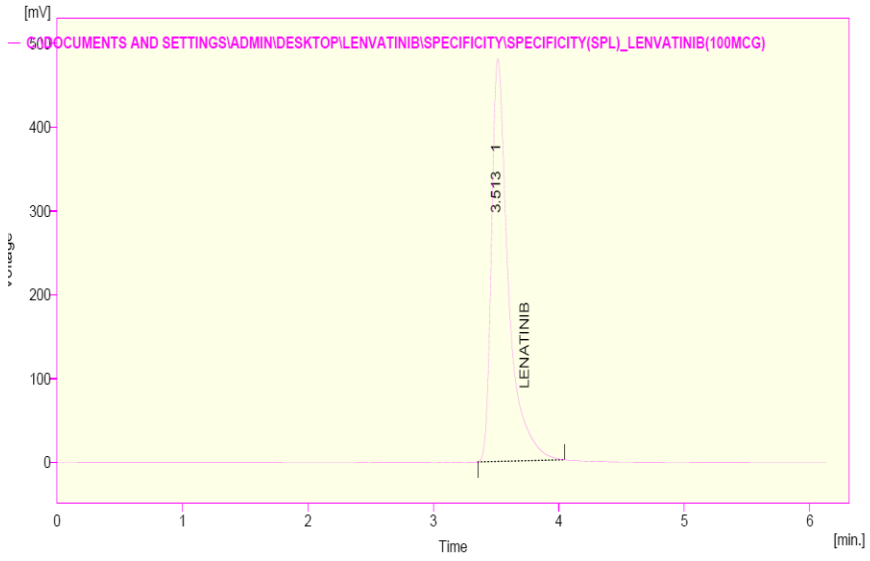


Fig: Chromatogram for specificity of LENVATINIB sample

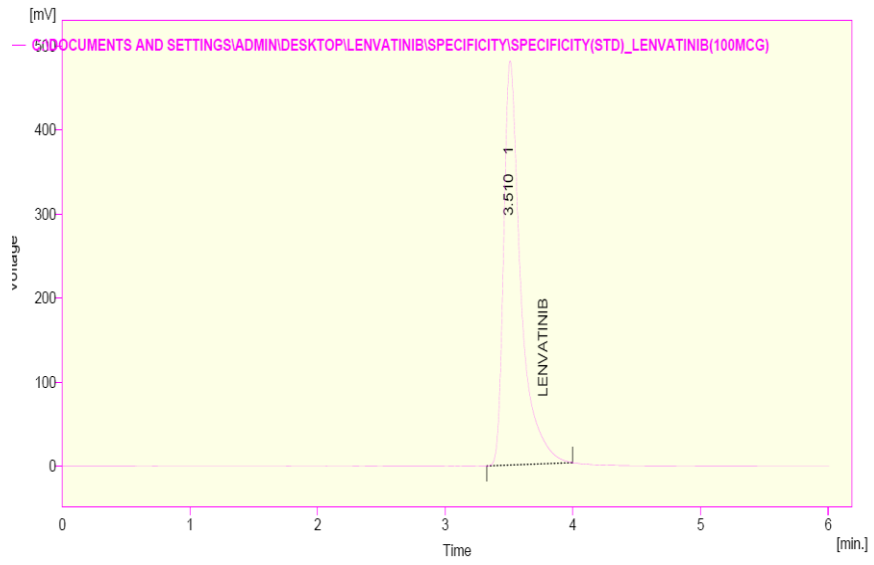


Fig: Chromatogram for Specificity of LENVATINIB standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the LENVATINIB peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of LENVATINIB (microgram/ml) were prepared by dissolving 10 mg and LENVATINIB dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. Further dilutions were given in the table

Table: Linearity Preparations

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)
Preparation 1	0.6	10	60
Preparation 2	0.8	10	80
Preparation 3	1.0	10	100
Preparation 4	1.2	10	120
Preparation 5	1.4	10	140

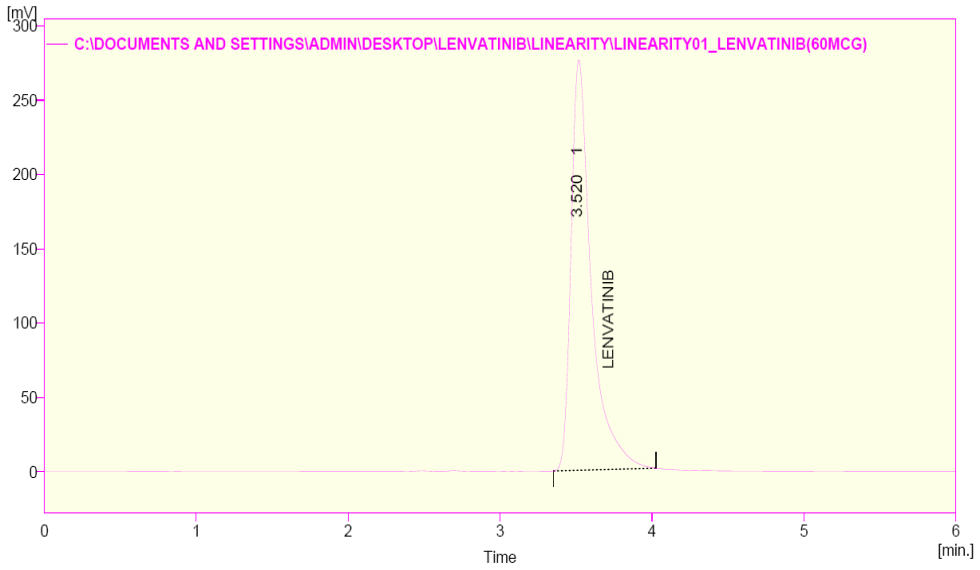


Fig: Chromatogram of LENVATINIB preparation-1

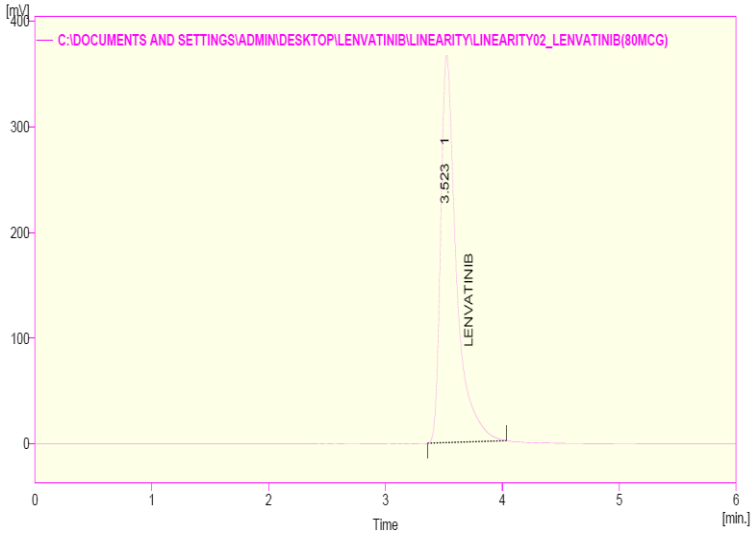


Fig: Chromatogram of LENVATINIB preparation-2

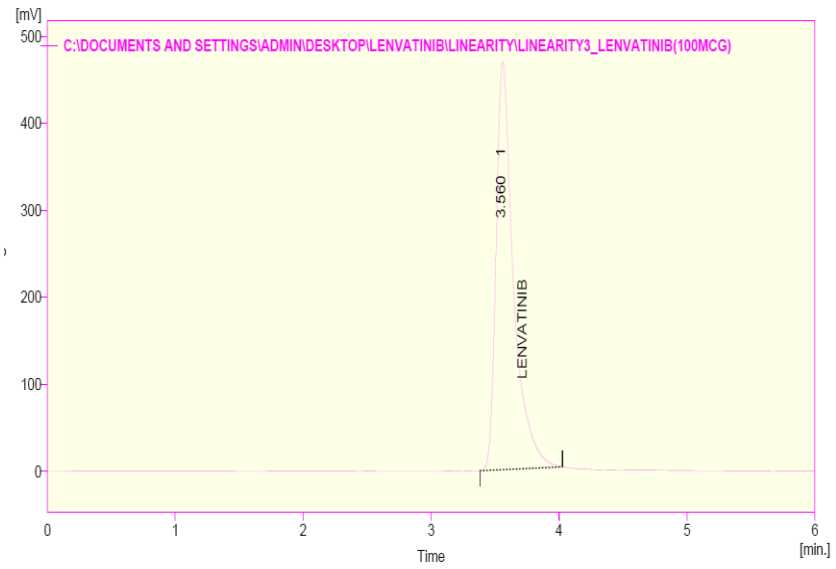


Fig: Chromatogram of LENVATINIB preparation-3

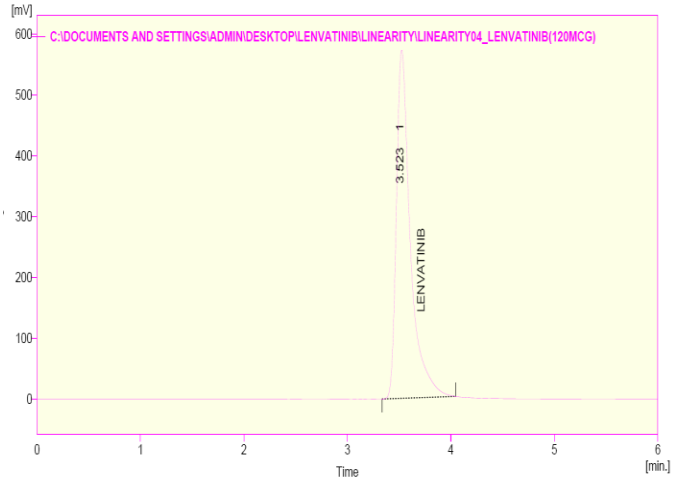


Fig: Chromatogram of LENVATINIB preparation-4

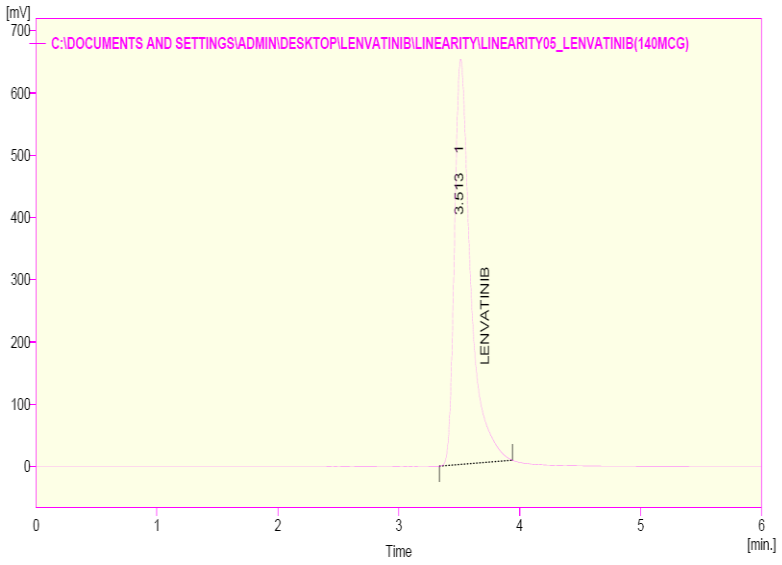
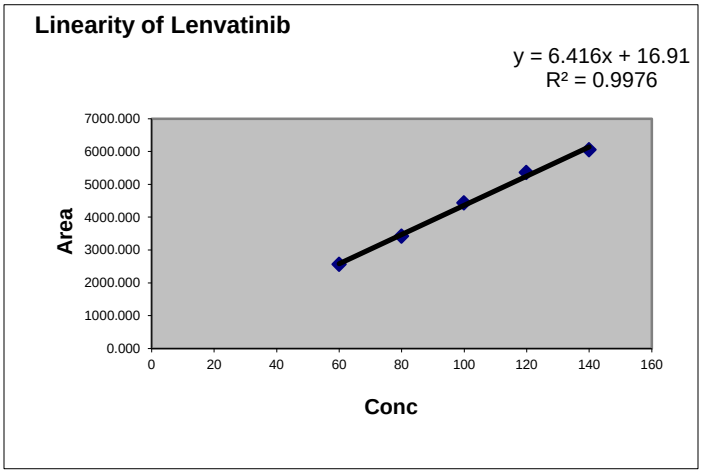


Fig: Chromatogram of LENVATINIB for preparation-5

Table: Linearity of LENVATINIB

PPM	AREA
60	2559.483
80	3414.608
100	4426.89
120	5354.686
140	6043.075

Linearity graph of LENVATINIB



Acceptance criteria

The relationship between the concentrations of LENVATINIB should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of LENVATINIB is 0.991 is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs 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 is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%

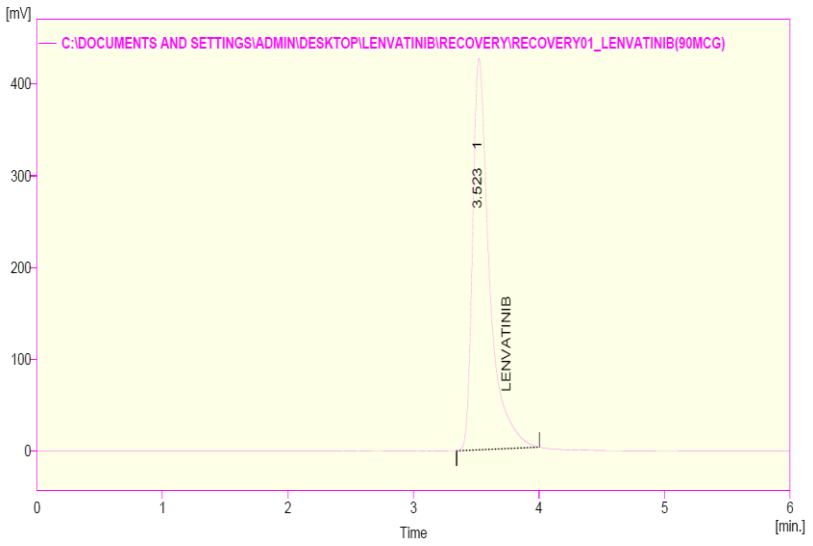


Fig: Chromatogram of 50% recovery (injection 1)

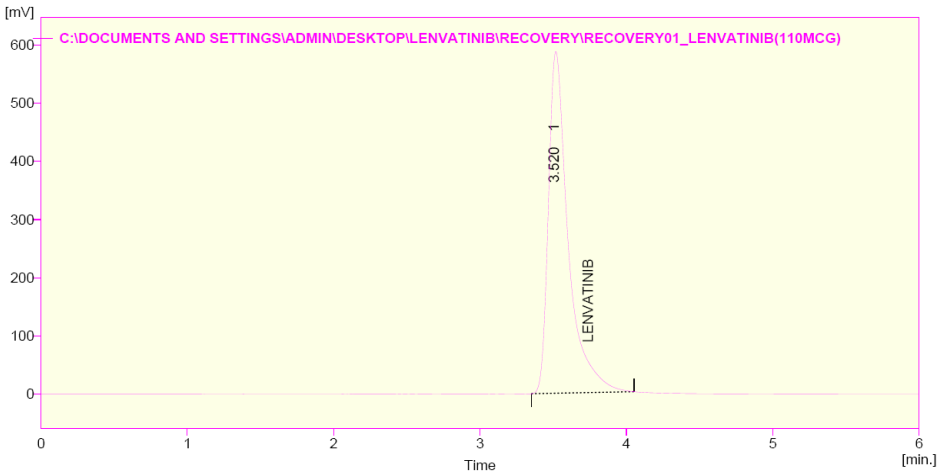


Fig: Chromatogram of 100% recovery (injection 2)

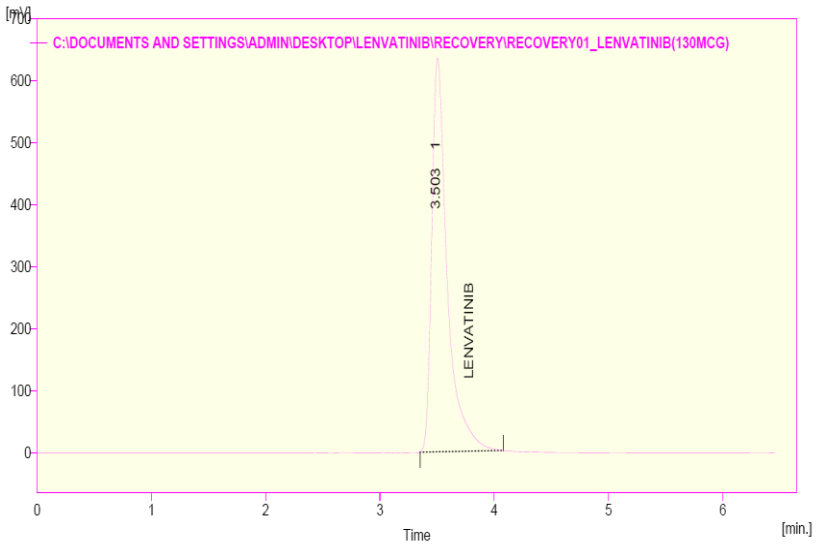


Fig: Chromatogram of 150% recovery (injection 3)

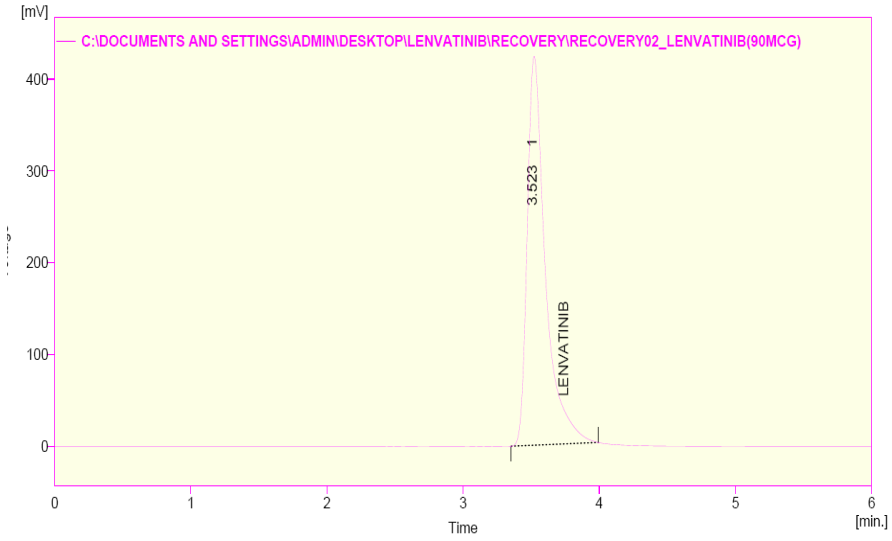


Fig: Chromatogram of 50% recovery (injection 1)

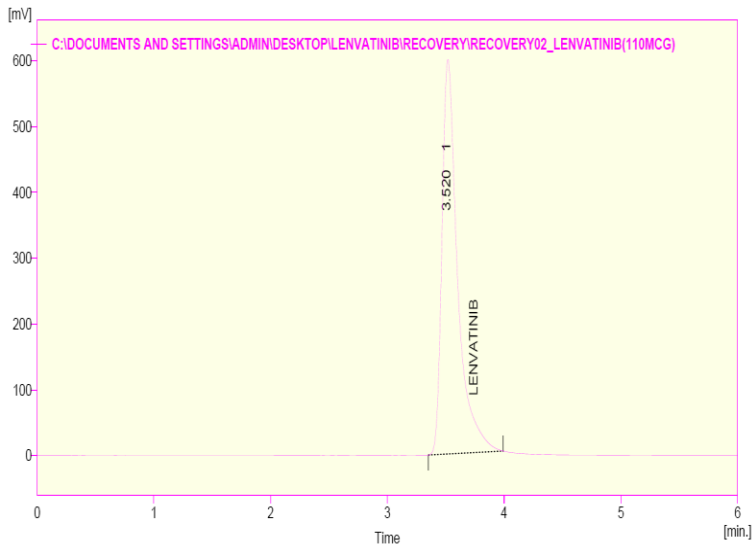


Fig: Chromatogram of 100% recovery (injection 2)

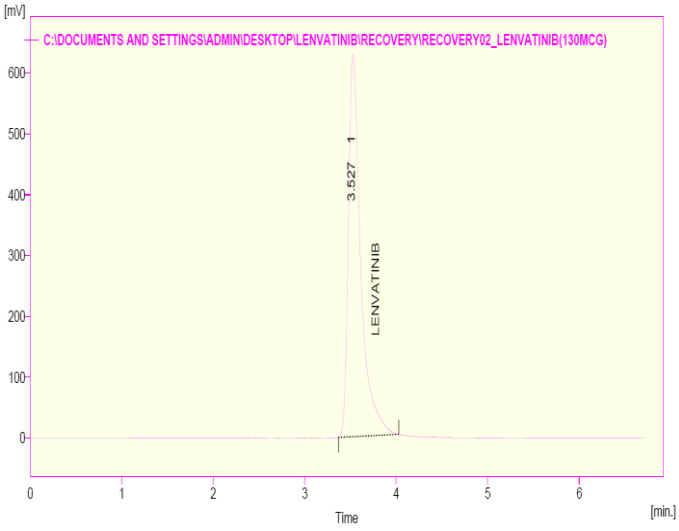


Fig: Chromatogram of 150% recovery (injection 3)

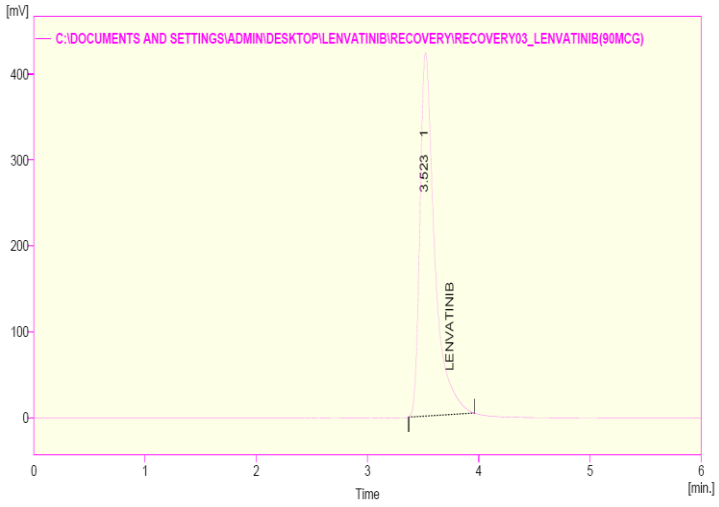


Fig: Chromatogram of 50% recovery (injection 1)

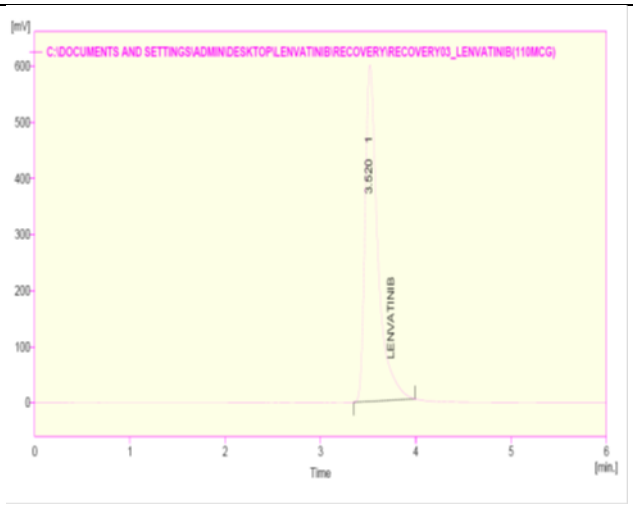


Fig: Chromatogram of 100% recovery (injection 2)

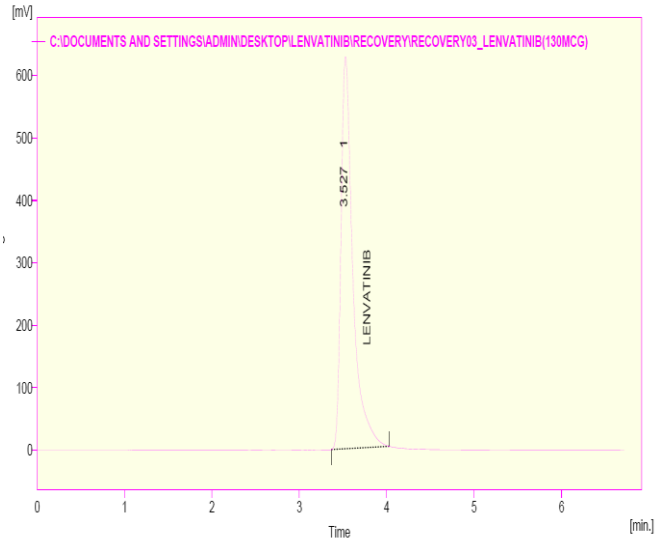


Fig: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of LENVATINIB should lie between 90% and 110%.

Table : Recovery results for LENVATINIB

Recovery level	Accuracy LENVATINIB				
	Amount taken(mcg/ml)	Area	Average area	%Recovery	Average % Recovery
75%	100	**4198.222**	**4296.968**	**100.67**	
	100	**4295.484**			
	100	**4397.199**			
100%	120	**5450.032**	**5464.189**	**102.86**	
	120	**5466.67**			
	120	**5476.267**			**100.5**
125%	140	**6195.931**	**6187.917**	**99.05**	
	140	**6184.41**			
	140	**6183.410**			

Precision

Method precision

Prepared sample preparations of LENVATINIB as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of LENVATINIB should be not more than 2.0%.

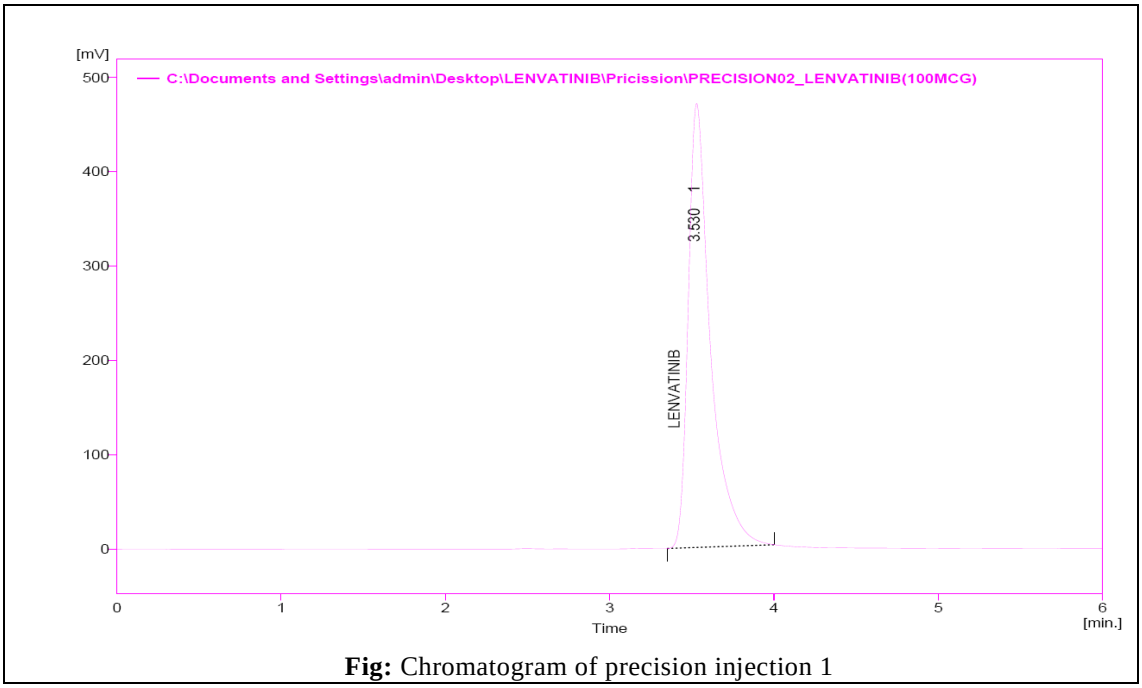


Fig: Chromatogram of precision injection 1

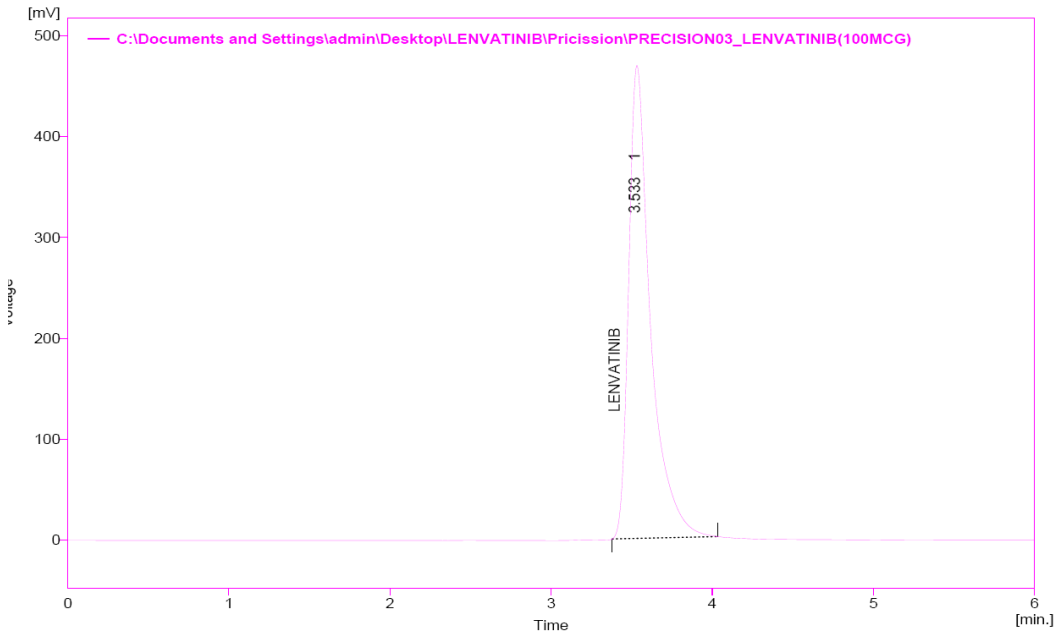


Fig: Chromatogram of precision injection 2

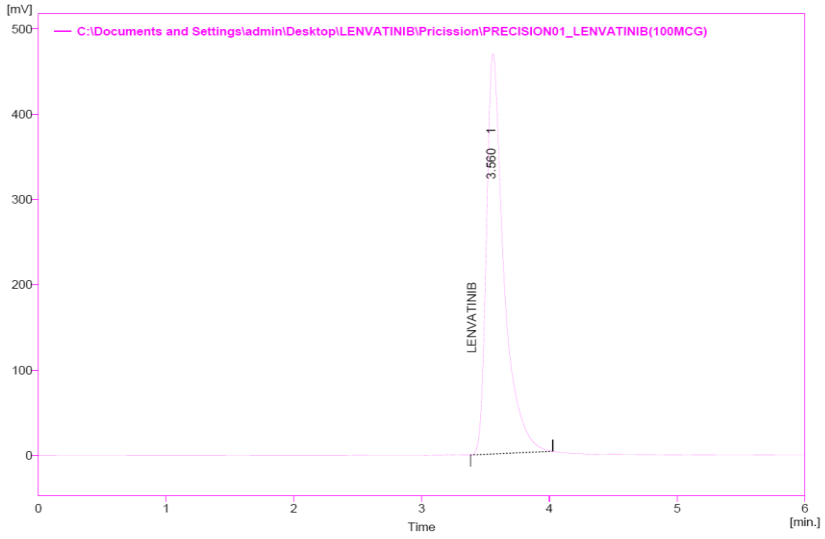


Fig: Chromatogram of precision injection 3

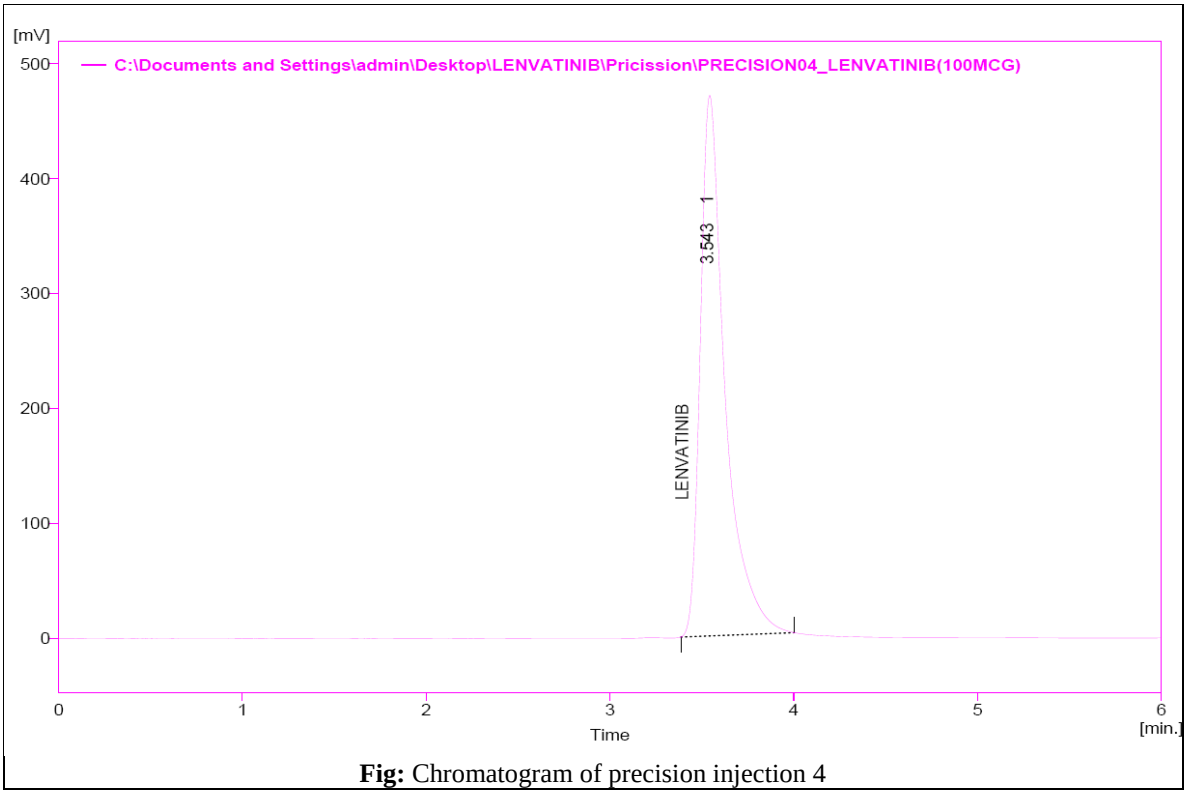


Fig: Chromatogram of precision injection 4

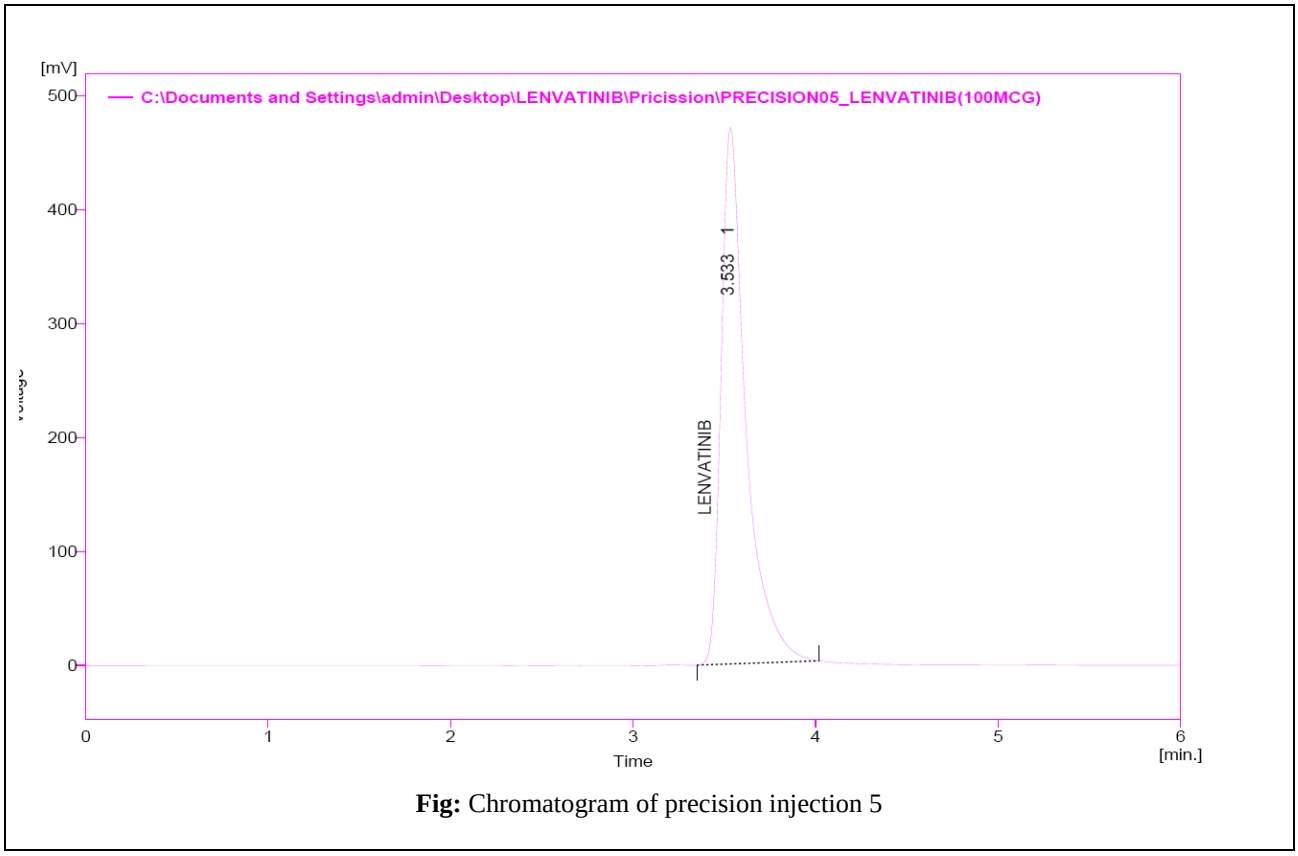


Fig: Chromatogram of precision injection 5

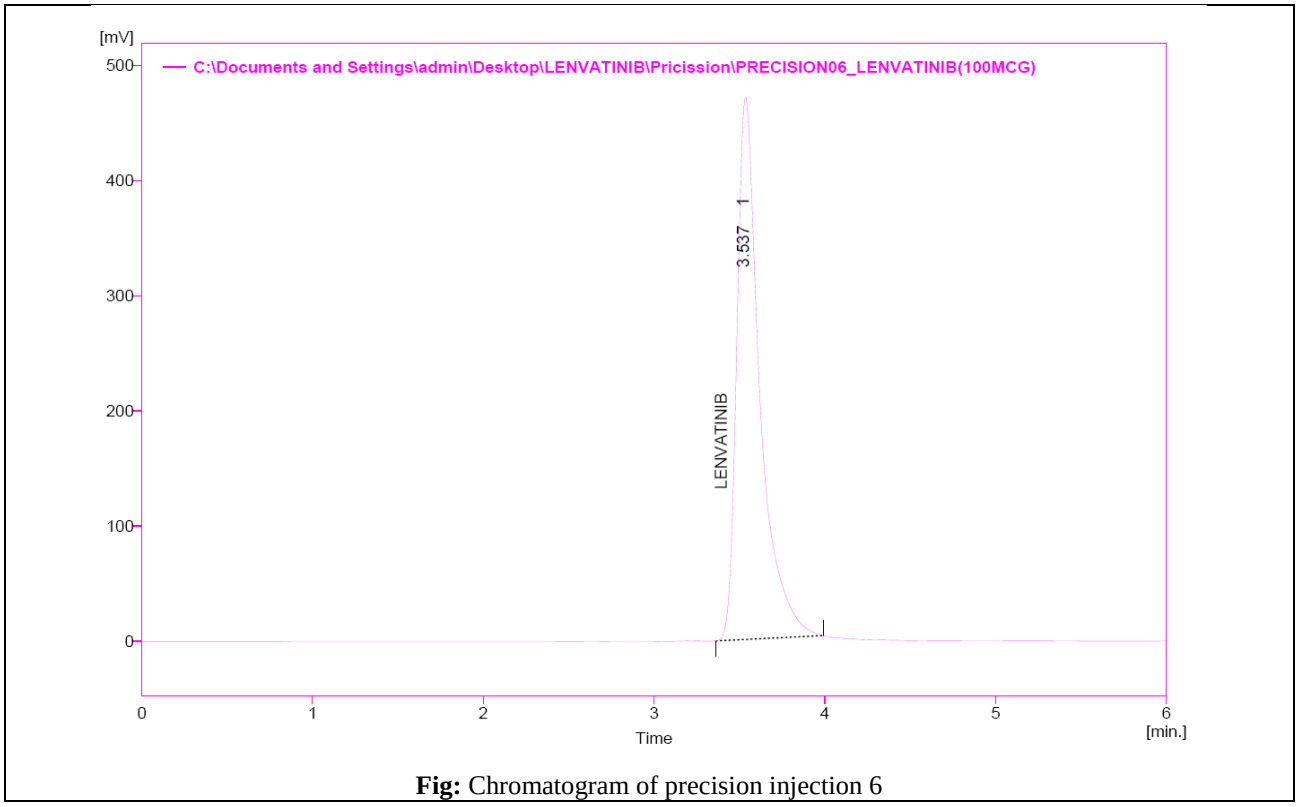


Fig: Chromatogram of precision injection 6

Table: Results for precision of LENVATINIB

Lenvatinib		
S.No.	Rt	Area
1	3.560	4426.890
2	3.530	4417.702
3	3.533	4410.326
4	3.543	4411.818
5	3.533	4442.974
6	3.537	4413.064
avg	3.5393	4420.462
stdev	0.0111	12.553
%RSD	**0.31**	**0.28**

Observation

Test results for LENVATINIBwas showing that the %RSD of Assay results are within limits.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

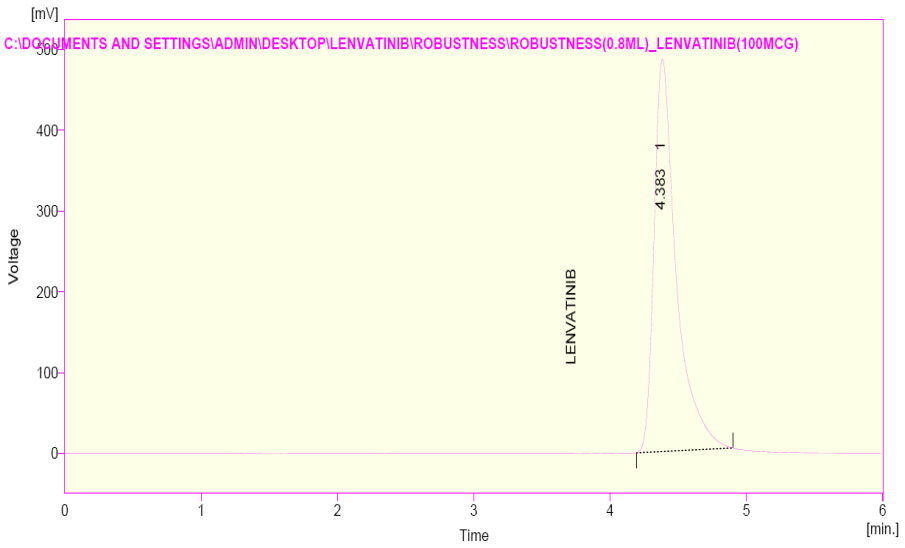


Fig: Chromatogram of LENVATINIB Robustness (0.8 ml/min)

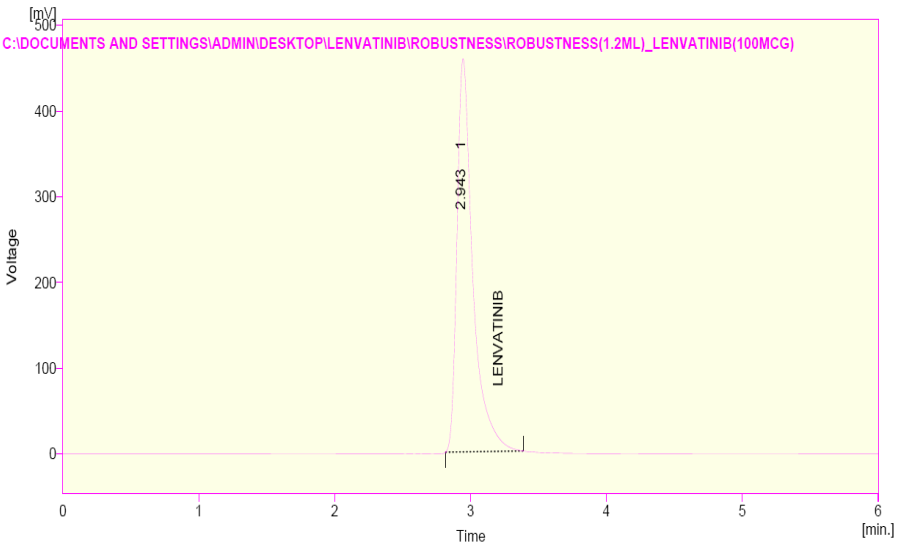


Fig: Chromatogram of LENVATINIB for Robustness (1.2 ml/min)

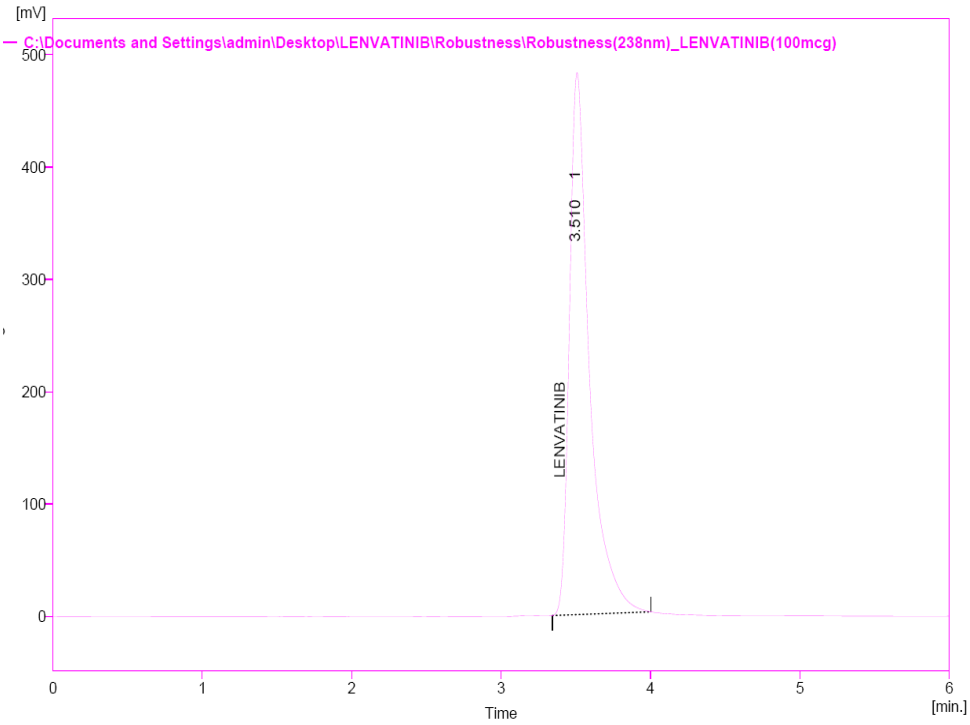


Fig: Chromatogram of LENVATINIB for Robustness (238nm)

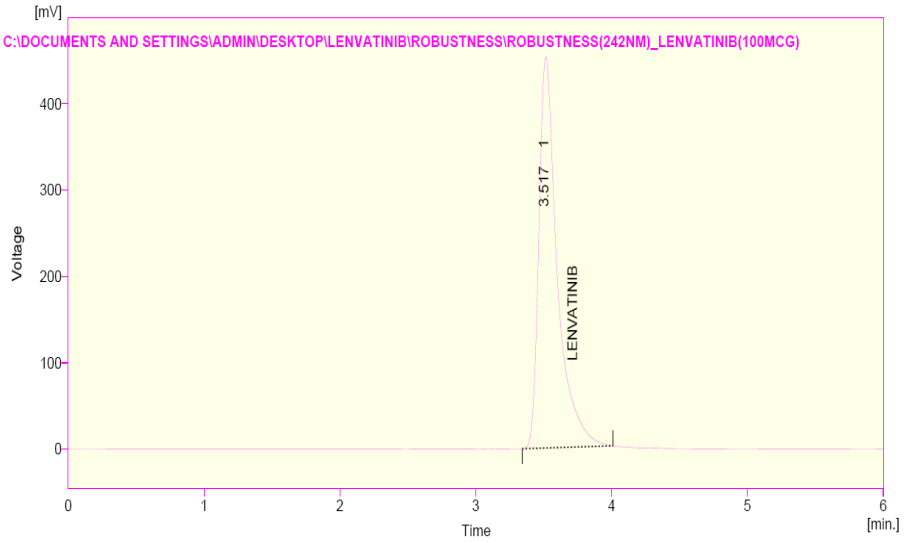


Fig: Chromatogram of LENVATINIB for Robustness (242nm)

Table: Result of Robustness study

Parameter	Lenvatinib	
	Retention time(min)	**Area**
Flow		
0.8ml/min	4.383	5477.973
1.2ml/min	2.943	3702.038

Wavelength		
238nm	3.510	4496.216
242nm	3.517	4253.798

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

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

Acceptance criteria

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

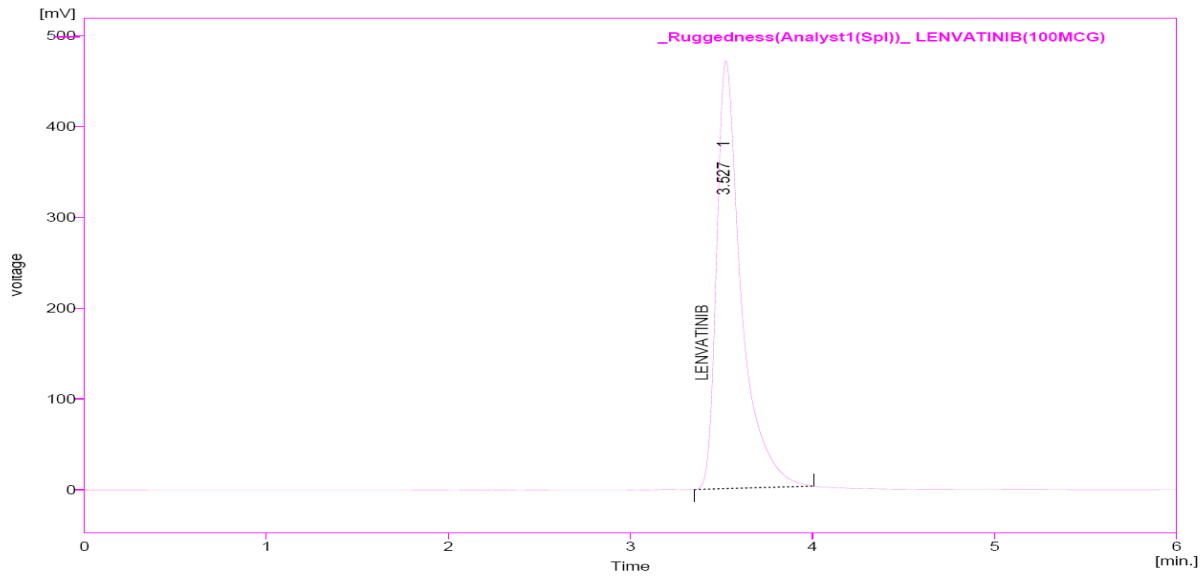


Fig: Chromatogram of Analyst 01 standard preparation

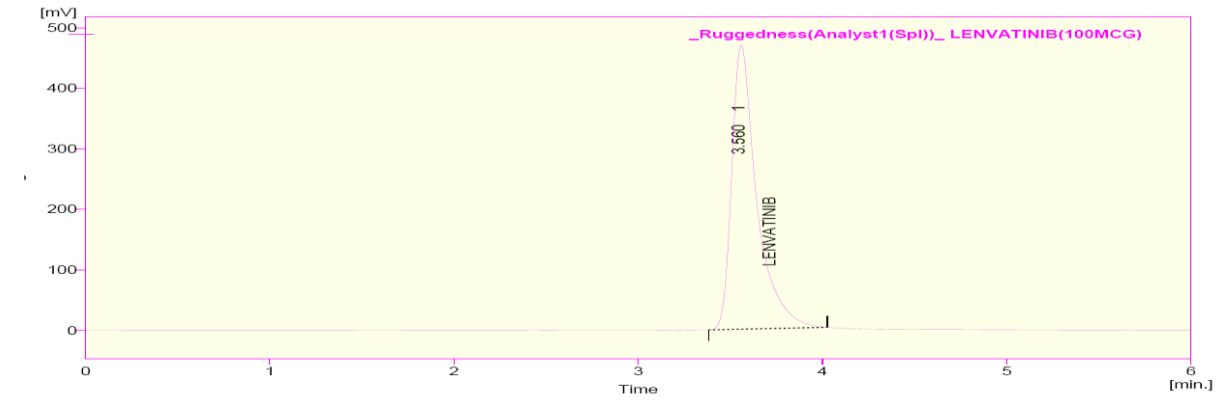


Fig: Chromatogram of Analyst 01 sample preparation

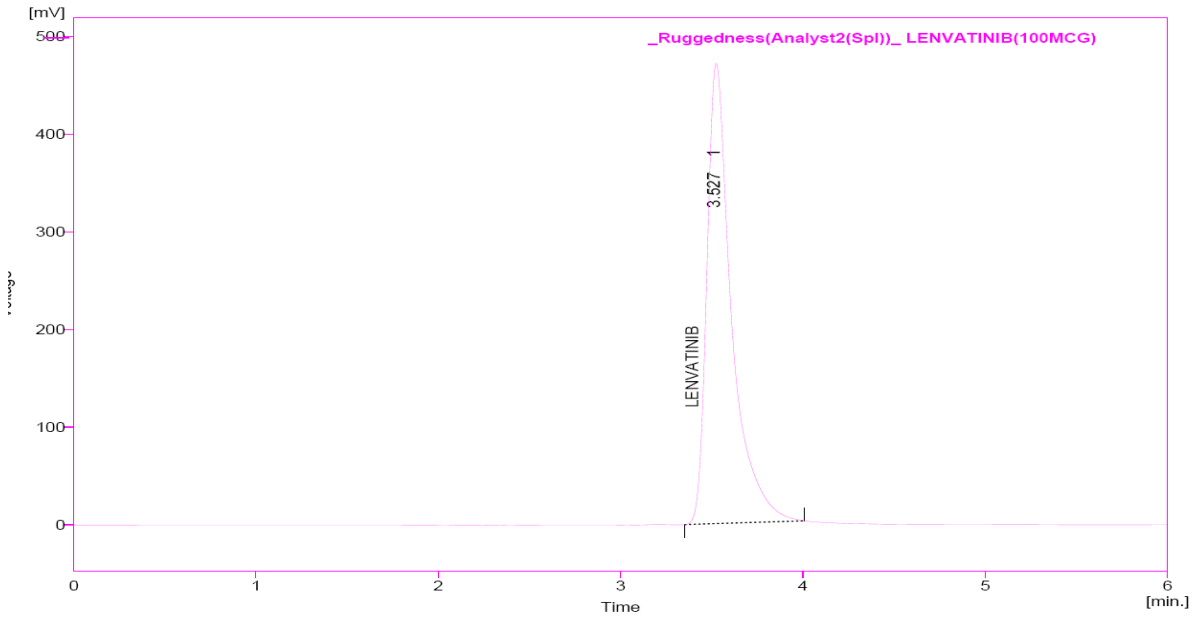


Fig: Chromatogram of Analyst 02 standard preparation

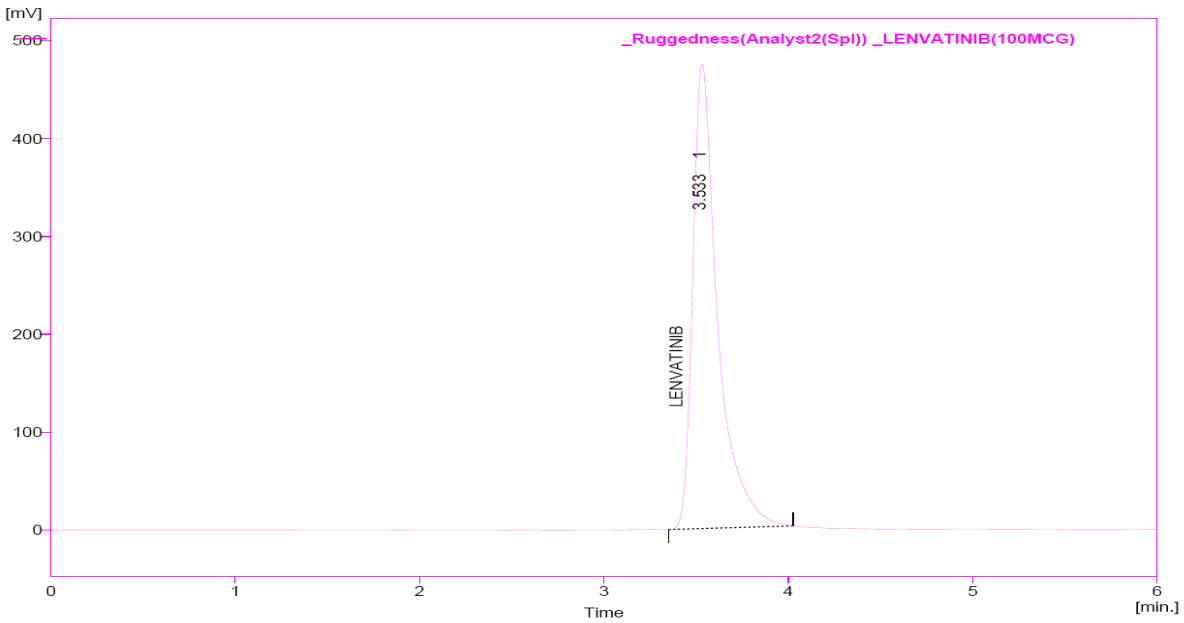


Fig: Chromatogram of Analyst 02 sample preparation

Table: Results for Ruggedness

Lenvatinib	%Assay
Analyst 01	99.92
Analyst 02	99.33
%RSD	0.27

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

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