Pradip Kumar Yadav
M Pharmacy, Department of
Pharmaceutical Analysis,
Aditya Bangalore Institute of
Pharmacy Education and
Research, Kogilu, Bangalore,
Karnataka, India

Paul Richards M
Professor, Department of
Pharmaceutical Analysis,
Aditya Bangalore Institute of
Pharmacy Education and
Research, Kogilu, Bangalore,
Karnataka, India

BA Vishwanath
Chairman, Department of
Pharmaceutical Analysis,
Aditya Bangalore Institute of
Pharmacy Education and
Research, Kogilu, Bangalore,
Karnataka, India

Analytical method for determination of Lenvatinib in pharmaceutical and bulk dosage form by using RP-HPLC method

Pradip Kumar Yadav, Paul Richards M and BA Vishwanath

Abstract
A new method was established for estimation of Lenvatinib by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lenvatinib by using thermosil C18 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 4.35 mins. The % purity of Lenvatinib was found to be 99.87%. The system suitability parameters for Lenvatinib such as theoretical plates and tailing factor were found to be 4146, 1.23, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Lenvatinib was found in concentration range of 30 µg-150 µg and correlation coefficient (r2) was found to be 0.999, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatability. LOD value was 2.97 and LOQ value was 9.92 Hence the suggested RPHPLC method can be used for routine analysis of Lenvatinib in API and Pharmaceutical dosage form.

Keywords: Lenvatinib, RP-HPLC method

Introduction
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, Tumor growth, and cancer progression in addition to their normal cellular functions, including fibroblast growth factor (FGF) receptors FGFR1, 2, 3, and 4.

Review of literature
1. A. Sreedevi *et al*, A simple, accurate, precise RP-HPLC method was developed and validated for the estimation of Lenvatinib in bulk and pharmaceutical dosage forms. The flow rate was maintained at 1.1 mL/min and effluents were monitored at 300 nm. The retention time was 3.164 min.
2. Arun kumar kalekar *et al*, An isocratic reverse phase liquid chromatography (RPHPLC) method has been developed and subsequently validated for the determination of Lenvatinib in its bulk and pharmaceutical dosage form seperation was achieved with a cosmicsil BDS C18;150 x 4.6 mm,particle size 5 micro meter and triethylamine buffer: Methanol and Acetonitrile as eluent at flow rate 1.0mL/min and the column temperature was 35 °C.UV detection was performed at 315nm.The method is simple, rapid, and selective.
3. Thulase Nadh Reddy. Dodda *et al*, an isocratic reverse phase High Performance liquid chromatography (RP-HPLC) method has been developed, subsequently validated and Stability indicating for the determination of Dasatinib and Lenvatinib in pharmaceutical.

Materials and Methods
Chemicals and Standard Used
1. Water Merck HPLC 2. Methanol Merck HPLC 3. Acetonitrile Merck HPLC 4. Ortho phosphoric acid Merck G. R 5. KH2PO4 Merck G. R 6. K2HPO4 Merck G. R 7. 0. 22µ Nylon filter Advanced lab HPLC 8. 0.45µ filter paper Millipore HPLC 9. In - House In-House.

Corresponding Author:
Pradip Kumar Yadav
M Pharmacy, Department of
Pharmaceutical Analysis,
Aditya Bangalore Institute of
Pharmacy Education and
Research, Kogilu, Bangalore,
Karnataka, India

Instrument Used

HPLC-auto sampler –UV detector Separation module2695, PDA detector Empower software version2 Waters. 2)U.V double beam spectrometer UV 3000 U.V win software Lab India 3)Digital weighing balance (sensitivity 5mg) ER 200A - Ascoset 4) pH meter AD 102U - ADWA 5 Sonicator SE60US - Enertech

Chemicals and Reagents

Pharmaceutically pure sample of Sumatriptan drug has obtained from Awamedica Company. Methanol, Water and Acetonitrile was obtained from the local market for HPLC

Chromatographic Condition

Trial 1

Column: Nucleosil C18 4.6x150mm 5µm Mobile phase ratio: Me OH: H2O (20:80%v/v) Detection wavelength: 265 nm Flow rate: 1ml/min Injection volume: 20µl Column temperature: Ambient Auto sampler temperature: Ambient Run time: 10min Retention time: 2.042 min

Trial 2

Column: Zodiacsil C14.6x150mm 5µm Mobile phase ratio: MEOH: H2O (65:35%v/v) Detection wavelength: 265 nm Flow rate: 1ml/min Injection volume: 20µl Column temperature: Ambient Auto sampler temperature: Ambient Run time: 6.0 min Retention time: 2.195 mints

Trial 3

Column: KROMOSIL RPC84.5×150mm 5.0 µm Mobile phase ratio: MEOH: pH 4.5 buffer (70: 30% v/v) Detection wavelength: 265 nm Flow rate: 1.0ml/min Injection volume: 20µl Column temperature: Ambient Auto sampler temperature: Ambient Run time: 6.0mins Retention time: 4.341 mints

Trial 4

Column KromosilRPC184.6×150mm 5µm Mobile phase ratio: methanol: pH 4.5buffer (60:40% v/ v) Detection wavelength: 265 nm Flow rate: 1.0ml/min Injection volume: 20µl Column temperature: Ambient Auto sampler temperature: Ambient Run time: 10 min Retention time: 5.009 mints

Trial 5

Column: Thermosil C18 4.6×150mm 5.0µm Mobile phase ratio: methanol: water (65: 35 % v/v) Detection wavelength: 265 nm Flow rate: 1 ml/min Injection volume: 20µl Column temperature: Ambient Auto sampler temperature: Ambient Run time: 6.0 mins Retention time: 2.585

Preparation of Buffer and Mobile Phase

Selection of mobile phase

Water: Methanol (35:65)

Selection of wavelength: 10 mg of was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for lenvatinib.

Selection of column

- Heart of HPLC made of 316 grade stainless steel packed with stationary phase. Silica based columns with different cross linking's in the increasing order of polarity are as follows: ⇓------- Non-polar---------- moderately polar--------Polar----------→ C18< C8< C6< Phenyl < Amino <Cyano< Silica

- In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material.

- Column is selected based on solubility, polarity and chemical differences among analytes and Column selected: i.e., Zodiac silC18 column 150×4.6 mm 5.0 µm.

- Reasons: Better separation,

Preparation of the lenvatinib sample solution

Sample solution preparation 10 mg equivalent lenvatinib capsule powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 1ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Procedure: 20µL of the blank, standard and sample were injected into the chromatographic system and areas for the lenvantinib the peak was used for calculating the % assay by using the formulae.

Results and Discussion

The present investigation reported in the thesis was aimed to develop a new method development and validation for the estimation of Lenvatinib by RP-HPLC method. Literature reveals that there are no analytical methods reported for the estimation Lenvatinib by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the estimation of Lenvatinib in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400 nm. The overlay spectrum of Lenvatinib was obtained and the point of Lenvatinib showed absorbance's maxima at 265 nm.

The chromatographic method development for the estimation of Lenvatinib was optimized by several trials for various parameters as different column, flow rate and mobile phase; finally the following chromatographic method was selected for the separation and quantification of Lenvatinib in API and pharmaceutical dosage form by RP-HPLC method.

Table 1: Showing results for assay standard:

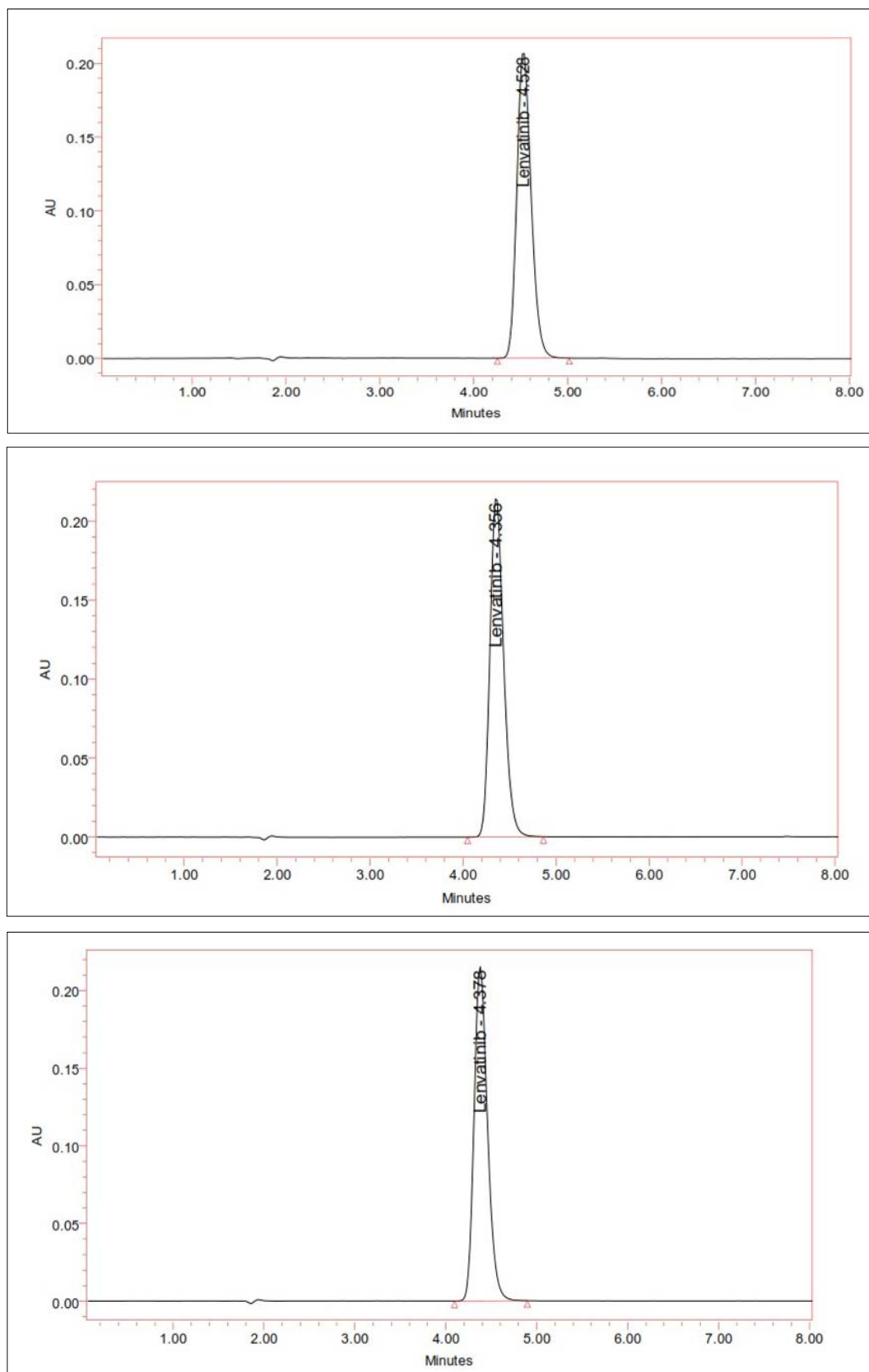|	Peak Name	RT	Area	Height	USP plate count	USP Taillig
1	Lenvatinib	4.372	2219524	220941	4398	1.2
2	Lenvatinib	4.368	2217869	220244	4386	1.2
3	Lenvatinib	4.361	2233932	223732	4425	1.2
Mean		4.4	2223775			
Std. dev.		0.0	8835.0			
%RSD		0.1	0.4			

Fig 1: Chromatogram showing assay of standard injections1 to 3

Linearity

The linearity study was performed for the concentration of 30 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The chromatograms are shown in Fig. and results are tabulated in Table. Calibration graph for Lenvatinib shown in Fig
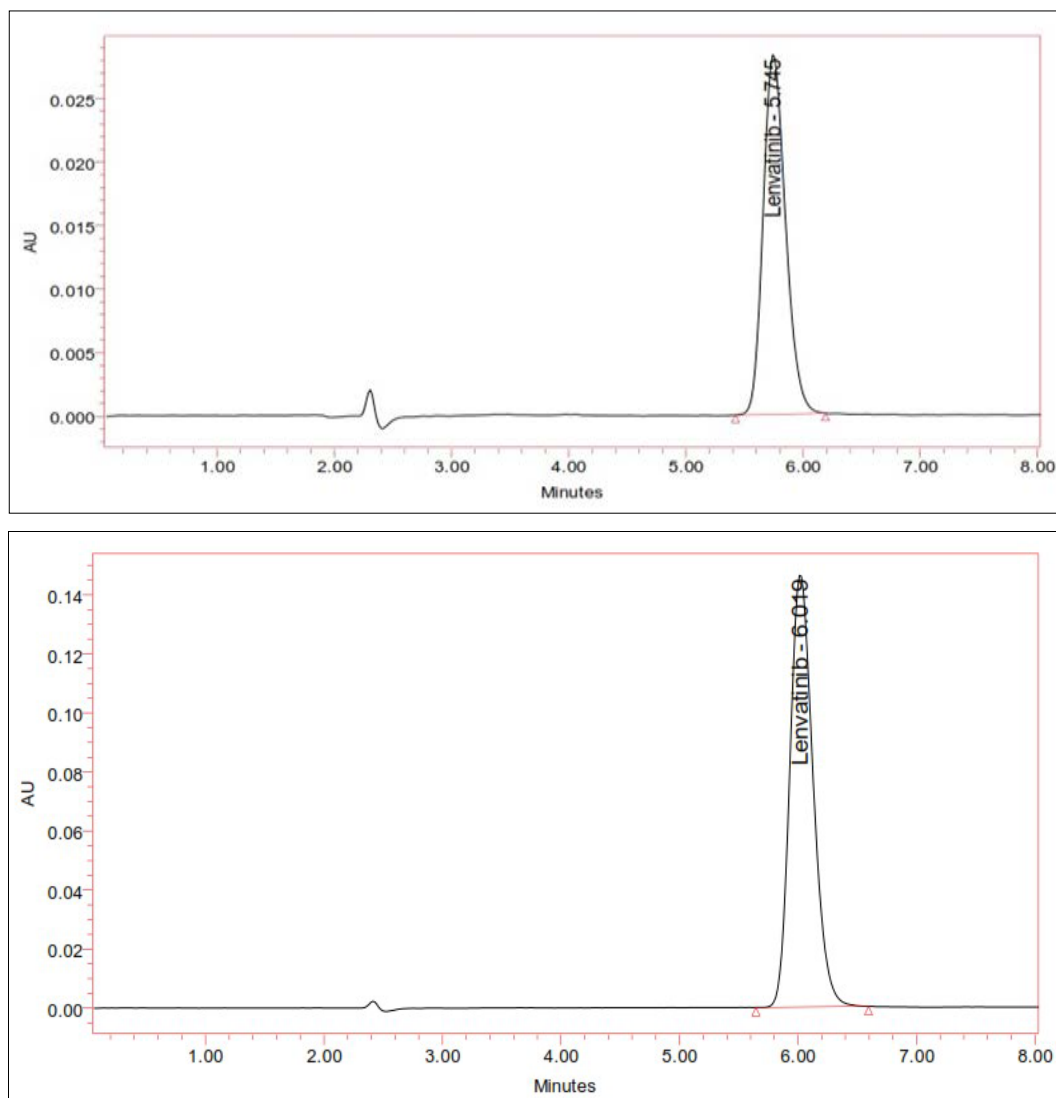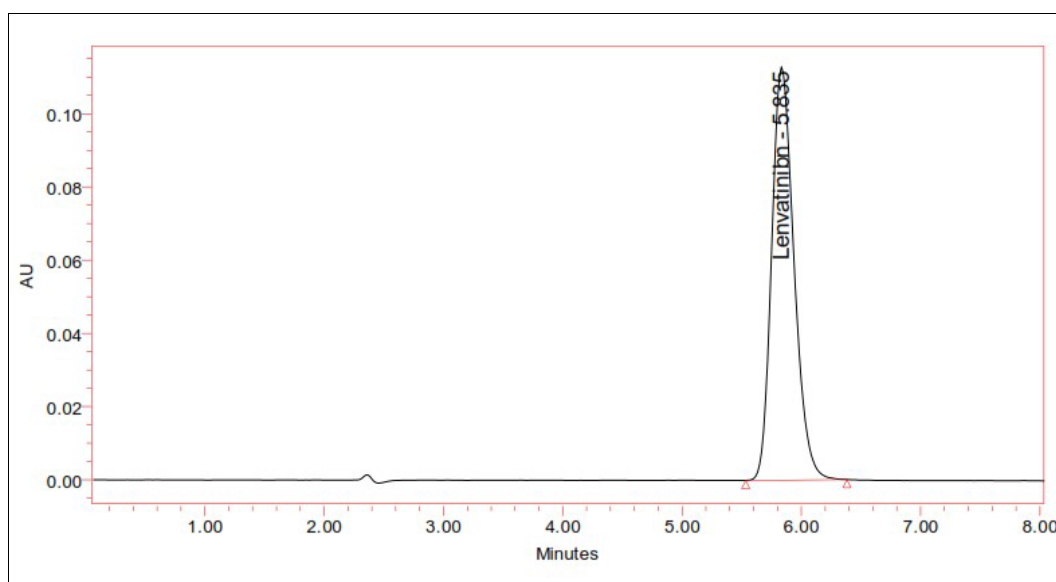
Fig 2: Chromatograms showing linearity level-1 to 5 (30 ppm to 150ppm)

Accuracy
The accuracy study was performed for 50%, 100% and 150 % for Lenvatinib. Each level was injected in triplicate into chromatographic system. The ar ea of each level was used for calculation of % recovery. Chromatograms are shown in Fig. and results are tabulated in Table.
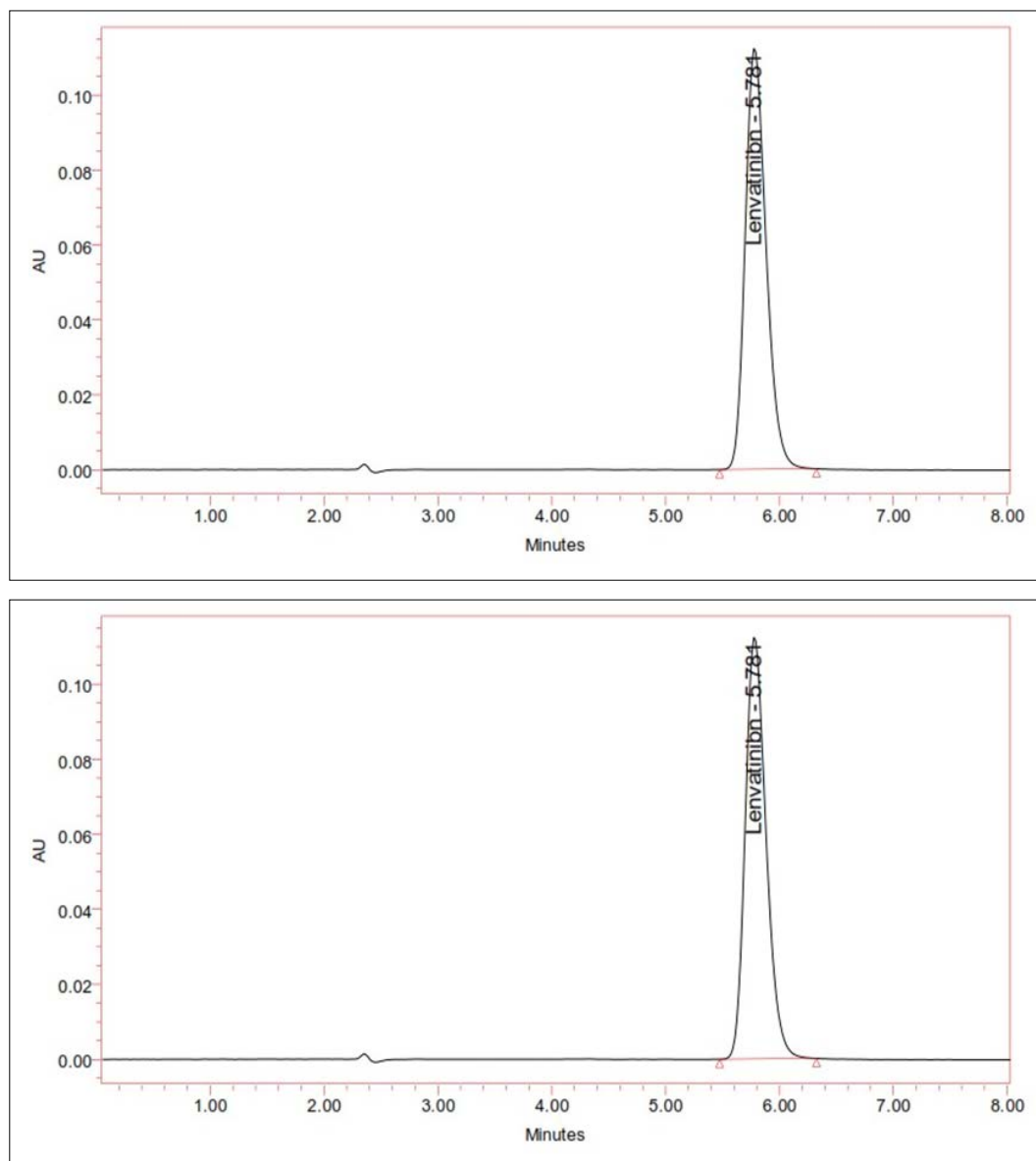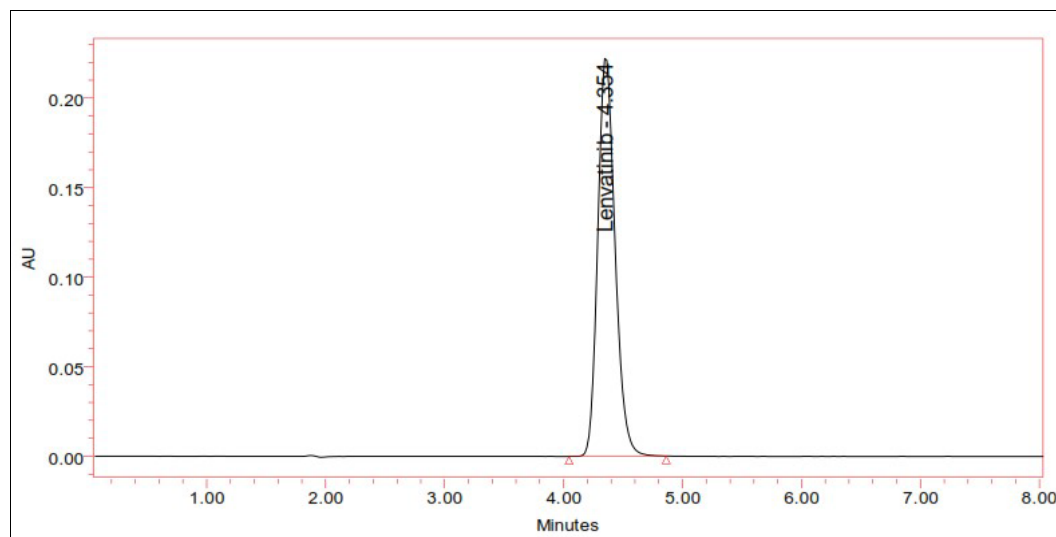
Fig 3: Chromatograms showing accuracy-50% injection-1,2,3.

Repeatability

The precision study was performed for five injections of Lenvatinib. Each standard injection was injected in to chromatographic system.

The area of each Standard injection was used for calculation of % RSD.

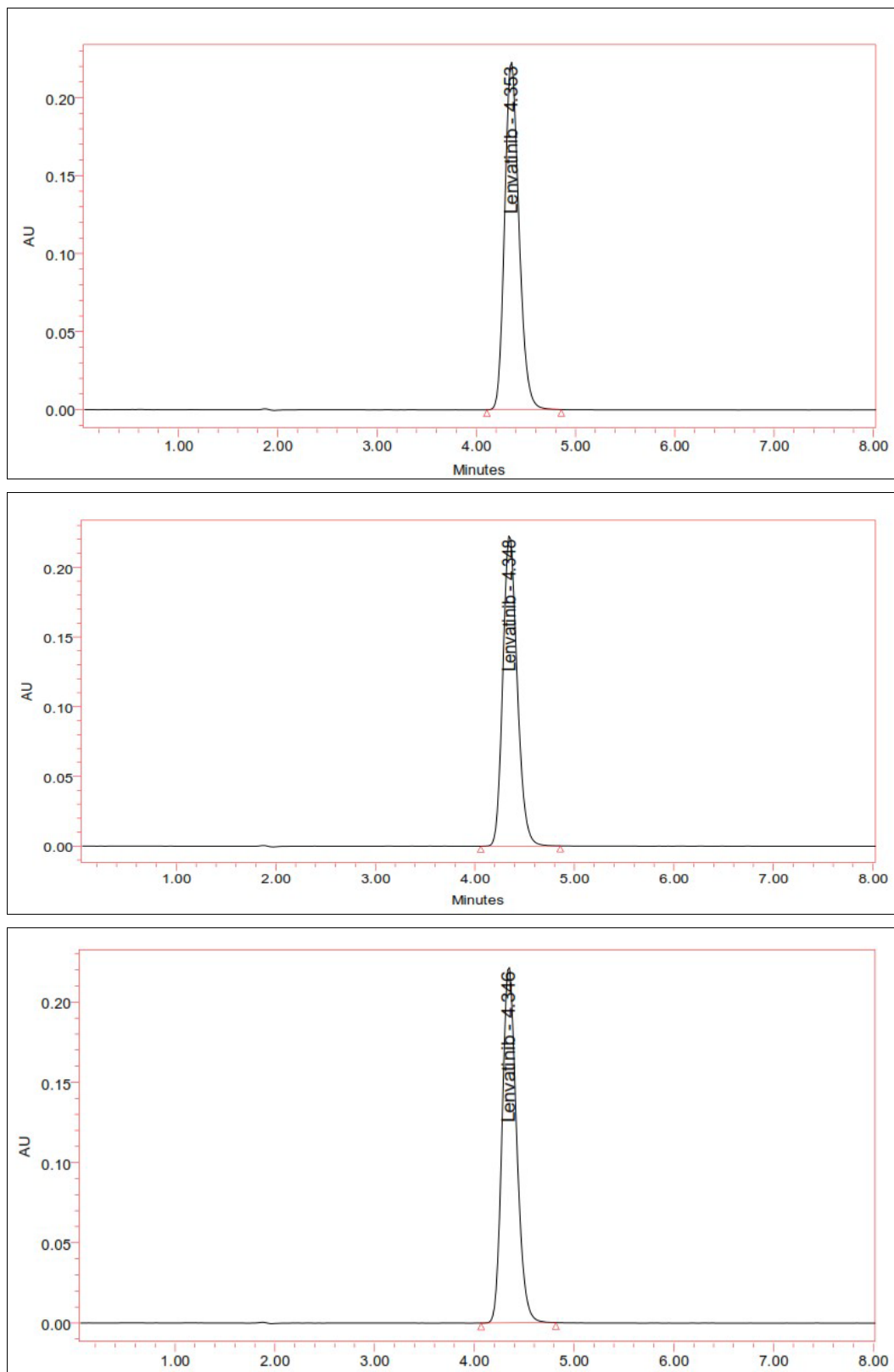The chromatograms are shown in Fig. and results are tabulated in Table.

Fig 4: Chromatogram showing precision injection -1 to 5.

Quantitation limit

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula.

Again, the standard deviation of the response can be determined based on the standard deviation of yintercepts of regression lines.
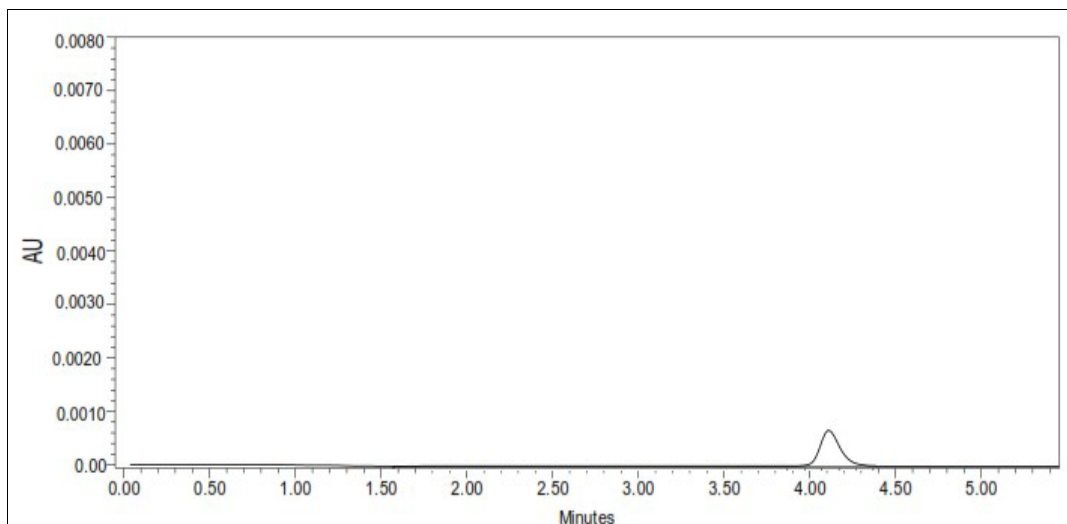
Formula:

$$LOQ = 10 \, X \, \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

Showing results for Limit of Quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Lenvatinib	371827.90	563365963	9.92

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Lenvantinib. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.The chromatograms are shown in Fig and results are tabulated in Table.
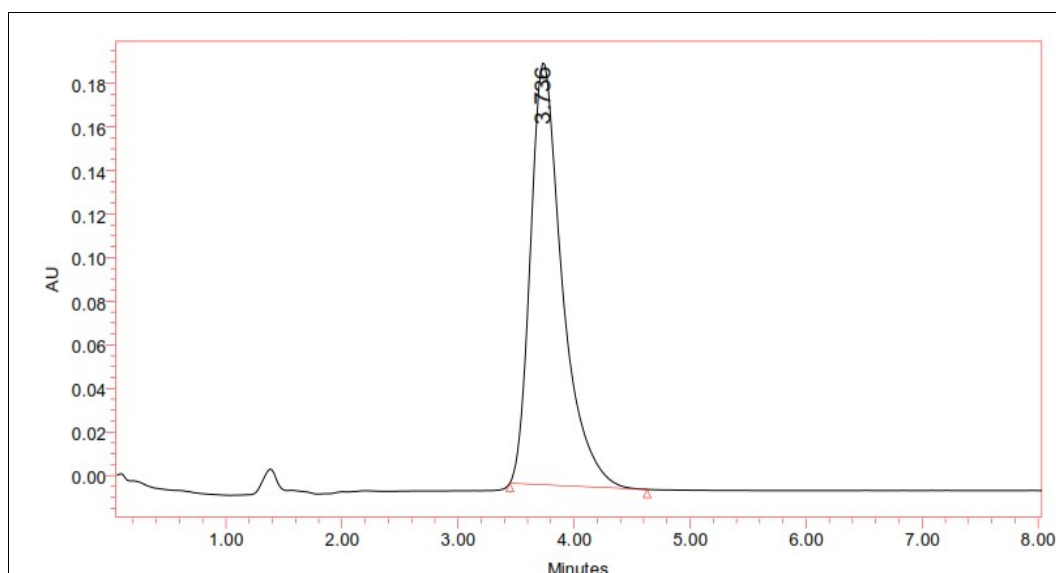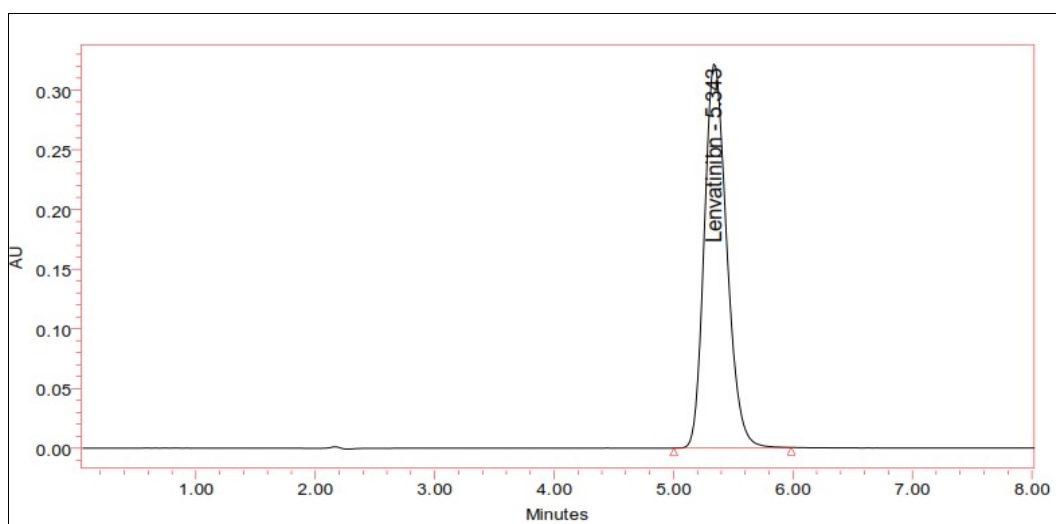
Summary and Conclusion

A new method was established for estimation of Lenvatinib by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lenvatinib by using thermosil C_{18} 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35% v/v methanol: water, detection wavelength was 265nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version2. The retention times were found to be 4.35 mins. The % purity of Lenvatinib was found to be 99.87%. The system suitability parameters for Lenvatinib such as theoretical plates and tailing factor were found to be4146, 1.23, the resolution was found to be 5.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Lenvatinib was found in concentration range of 30µg-150µg and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilIty. LOD value was 2.97 and LOQ value was 9.92.

Hence the suggested RP-HPLC method can be used for routine analysis of Lenvatinib in API and Pharmaceutical dosage form.

References

1. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication, Meerut; c2007.
2. Lindholm J. Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis; c2004. p. 13-14.
3. Rashmin. An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences. 2012;2(2):191196.
4. Malvia R, Bansal V, Pal OP, Sharma PK. A Review of High-Performance Liquid Chromatography. Journal of Global Pharma technology; c2010.
5. Douglas Skoog A, James Holler F, Timothy Niemen A. Principles of Instrumental Analysis, 725-760.
6. Ravi Shankar S. Text book of pharmaceutical analysis, Fourth edition, p. 13.1-13.2
7. David G. Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed, p. 221-232.
8. Remingtonn's The Sciences and Practise of Pharmacy, 20th Edition; c2000.