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Analytical Method Development And Validation For Determination Of Nilotinib By HPLC

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Abstract

The present study was focused to develop a simple, precise, accurate and cost effective HPLC method for estimation of Nilotinib from pharmaceutical dosage form. The chromatographic method was carried out using Younglin (S.K) Gradient System UV Detector, Detecter & pump No.UV 730 D & SP930 D,Autochro -3000 software,Column 4.6 x 250 mm, C-18 {4.6 x 250 mm (5µm)}Particle size packing 5 µm,Stationary phase C_{18} ,**C-18 {4.6 x 250 mm (5µm)},**Mobile phase Methanol (80) : Water(20) (0.1 % Acetic Acid).The flow rate was set 0.8 ml/min with 20 µl injection volume. Total run time was 4.71min Detection was carried out at wavelength of 260nm.The detector response was linear in the range of 5-25µg/ml .The % recoveries obtained in the accuracy study were 98.28-101.58% .The LOD was found to be 0.1198g/ml.The LOQ was found to be 0.3631g/ml

The developed method is successfully applied for estimation of Nilotinib from pharmaceutical dosage form.

Key Words:HPLC, Method development,Nilotinib,Tyrosine Kinase inhibitor,chronic myeloid leukaemia

I. INTRODUCTION

1.1 INTRODUCTION CHRONIC MYELOID LEUKAEMIA (CML)

It is a blood and bone marrow malignancy.

CML is typically detected during its chronic phase, when treatment is highly beneficial for the majority of patients.

CML is divided into three stages.

CML is also known as chronic myelogenous leukaemia, chronic granulocytic leukaemia, and chronic myelocytic leukaemia. CML is one of the four most common kinds of leukaemia.

Haematologists and oncologists are medical doctors who treat patients with CML and other kinds of blood cancer.

The majority of CML patients are given daily oral medication therapy.

CML has been changed from a life-threatening disease to a manageable chronic condition for the vast majority of patients since the advent of tyrosine kinase inhibitor (TKI) therapy in 2001. People with CML are living longer lives and requiring fewer treatments.

1.1.1 How Does CML Grow?

A growing stem cell's DNA (genetic material) in the bone marrow gets destroyed. This is referred to as a "acquired mutation."

This injured cell develops into a leukemic cell and multiplies into a large number of CML cells. CML cells proliferate and survive longer than normal cells.

As a result, the number of healthy blood cells (red blood cells, white blood cells, and platelets) is often lower than normal.

Errors can occur during the process of a cell replicating itself or dividing into new cells. One sort of error is known as a "translocation." When a fragment of one chromosome breaks off and joins to another, this is referred to as a translocation. This can result in a "fusion gene," which is an aberrant gene generated when two distinct genes combine.

The BCR::ABL1 fusion gene is responsible for all occurrences of CML. Normal blood cells do not include this gene.

The BCR::ABL1 gene is produced during cell division by a translocation between portions of chromosomes 9 and 22 in a single bone marrow cell.The "Philadelphia chromosome" (Ph chromosome) is the name given to the defective chromosome 22.

Genes encode instructions for cells to produce proteins.The BCR::ABL1 oncogene generates an aberrant protein known as "BCR::ABL1 tyrosine kinase," which causes CML cells to grow.

Risk factors

Most persons who acquire chronic myeloid leukaemia (CML) have no evident cause for the disease. CML is not inherited. It occurs when the DNA of a single bone marrow cell is damaged. CML risk factors include:
CML is somewhat more frequent in men than in women.
Age - The chance of developing CML rises with age.
Radiation exposure - CML is produced in a tiny percentage of individuals by exposure to extremely high amounts of radiation (for example, being a survivor of an atomic bomb blast or a nuclear reactor accident).
Some people who get high-dose radiation therapy for other malignancies, such as lymphoma, see a modest increase in risk. The majority of individuals who are treated for cancer with radiation do not get CML, and the majority of persons who do have CML have not been exposed to large doses of radiation.
Exposure to diagnostic dentistry or medical X-rays has not been linked to an increased incidence of CML. CML has been documented in people who had too many diagnostic X-rays or computed tomography (CT) scans, thus each X-ray and CT scan must be properly justified to reduce the risk of CML and other kinds of leukaemia..

1.2 Tyrosene kinase inhibitor therapy

Targeted treatment includes tyrosine kinase inhibitors (TKIs). TKIs are administered orally as tablets. A targeted treatment recognises and targets certain types of cancer cells while inflicting minimal harm to healthy cells. TKIs in CML target and prevent the action of the aberrant BCR::ABL1 protein, which promotes uncontrolled CML cell proliferation, causing the CML cells to perish.

The first treatment for an illness is referred to as "initial" or "first-line" treatment. The four TKI medicines listed below are authorised as first-line therapy for chronic phase CML:

- Imatinib mesylate
- Dasatinib
- Nilotinib
- Bosutinib

Patients having a history of cardiac or peripheral vascular illness must be carefully and often evaluated throughout TKI therapy. TKIs have caused substantial cardiac adverse effects in certain individuals, including heart attacks and abnormalities in pulse rhythm. Some have acquired constriction of the arteries in the brain's extremities, which can lead to a stroke. Many individuals who acquire these side effects often have other health issues and risk factors, such as advanced age, high blood pressure, high cholesterol levels, diabetes, or a history of heart illness, so careful monitoring is essential.

1.2.1 Drug Interactions

TKIs' physiological effects can be influenced by a variety of medications, natural remedies, and even diets. Some TKIs may be rendered ineffective by corticosteroids, anti-seizure drugs, antacids, and the herbal supplement St. John's Wort. Certain antibacterial, antifungal, and grapefruit products can raise the blood levels of TKIs to dangerously high levels.
TKIs may interact negatively or even fatally with other prescription drugs, over-the-counter drugs, dietary supplements, and even meals.To make sure the goods are safe to consume with their TKIs, patients should always give their doctors a list of all the drugs, herbal supplements, and vitamins they are taking. Additionally, it's crucial to ask the doctor whether there are certain foods to stay away from.

1.2.2 TKI Adherence

Patients should take their TKIs exactly as directed by their doctor. In order to be compliant with an oral therapy, a patient must: Take the drug at the right time; Never miss a dosage; Never take an additional dose.

Follows any additional instructions, such as taking the drug with a meal or glass of water. Does not take a dose with meals, beverages, supplements, or other medications that are not allowed.

Treatment-Free Remission

When a patient who has stopped receiving TKI therapy still has a major molecular response (MMR) and does not require treatment restart, this is known as a treatment-free remission (TFR).

Patients with chronic-phase CML who develop and sustain a stable, deep molecular response (DMR) for at least two years are thought to be suitable candidates for stopping TKI therapy, with appropriate medical supervision..

1.2.3 Why Consider TFR?

Treatment-free remission has a number of potential benefits for CML patients. These may combine aspects of the patient, the cost, and the treatment. Reducing the possibility of future TKI adverse effects and drug interactions is one benefit of quitting treatment. TKIs normally have few side effects, although some of them can have a negative impact on health and quality of life.

Benefit young female patients who are planning to have children and may require treatment breaks

Reduce costs for both patients and the healthcare system by eliminating patient co-pays and insurance fees for ongoing treatment. Reduce the difficulty of taking daily medication**.**

1.2.4 TKI Withdrawal Syndrome.

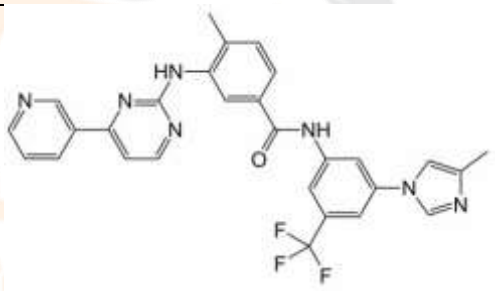
Some patients may experience rash or joint and/or muscle pain after stopping TKI therapy. In general, over-the-counter pain relievers can be used to treat the pain.
There have been reports of TKI withdrawal syndrome in 10 to 30% of patients who stopped taking TKIs.

Patient Concerns. The main anxiety that patients experience regarding stopping TKI therapy is fear of recurrence or progression of CML. Ask questions and ask for additional information. Make sure all questions are answered before making a decision

2 Drug profile

This compound belongs to the class of organic compounds known as n- phenylbenzamides, benzamides are N-linked to a phenyl group via the carboxamide group

Table 1 **Drug profile**

Sr.No	Parameter	Description
1.	Structure of Nilotinib	
2	CAS No	641571-10-0
3.	Drug Category	Anticancer
4.	Chemical Formula	$C_{28}H_{22}F_3N_7O$
5.	Molecular Weight	529.5158g/mol
6.	IUPAC Name	4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}benzamide
7.	State and appearance	Solid ,slightly yellowish
8.	Solubility In Solvents	Soluble in Acetonitrile ,Methanol .Water etc
9.	Routes	Oral

2.1 Mechanism of action

The BCR-ABL oncogene is the primary cause of chronic myelogenous leukaemia (CML). The BCR-ABL protein's tyrosine kinase activity is inhibited by nilotinib. Higher affinities of nilotinib than imatinib allow it to bind to the ATP-binding site of the BCR-ABL protein and overcome mutation-based resistance. The potential use of AMN107 for myeloproliferative diseases characterised by these kinase fusions is suggested by its inhibition of TEL- platelet-derived growth factor receptor-beta (TEL-PDGFR beta) and FIP1-like-1-PDGFR alpha; it also inhibits the c- Kit receptor kinase, including the D816V-mutated variant of

KIT, at pharmacologically achievable concentrations, supporting potential utility in the treatment of mastocytosis, and gastrointestinal stromal tumours

2.2 Pharmacodynamics

The transduction inhibitor nilotinib, which may be used to treat a variety of leukaemias, including chronic myeloid leukaemia (CML), targets BCR-ABL, c-kit, and PDGF.

2.3 Adverse Reactions

Many of the negative effects of nilotinib are common for anti-cancer medications. These include flu-like symptoms, low blood cell count, headache, exhaustion, gastrointestinal issues like nausea, vomiting, diarrhoea, and constipation, muscular and joint pain, rash, and other skin diseases. Cardiovascular adverse effects such hypertension (high blood pressure), various forms of arrhythmia, and a prolonged QT interval are less common. The electrolyte and glucose balance of the body may potentially be impacted by nilotinib. There is a case report of severe respiratory failure caused by diffuse alveolar haemorrhage in a patient on Nilotinib, despite the fact that pulmonary-related side effects are uncommon when compared with imatinib and dasatinib.

2.4 Side effects

Night sweats, muscular cramps, back, bone, joint, limb, or muscle discomfort, hair loss, dry or reddish skin, numbness, burning, or tingling in the hands or feet, among other symptoms, might make it difficult to fall asleep or stay asleep.

Shortness of breath, swelling of the hands, ankles, feet, or face, sudden stomach pain, yellowing of the skin or eyes, unusual bruising or bleeding, blood in the urine, tarry stools, fever, chills, sore throat, persistent cough and congestion, or other signs of infection are some side effects that can be serious.

2.5 Overdose

A variety of symptoms, such as fever, sore throat, chills, or other infection-related symptoms, vomiting, and tiredness, might result from an overdose.

2.6 Contraindication

Hypokalemia, hypomagnesemia, pregnancy, lactation, galactose/lactose intolerance, liver dysfunction, and pancreatitis history are examples of contraindications.

Nilotinib dose reduction has been suggested for populations with liver disease, and this entails recommending a lower starting dose and keeping an eye out for any changes in hepatic function.

2.7 Interaction

It has been noted that nilotinib is an OATP1B1 and OATP1B3 substrate. Nilotinib's hepatic distribution may be altered by interactions with OATP1B1 and OATP1B3, which may result in transporter-mediated medication interactions. Nilotinib blocks the OATP-1B1 transporter, but not the OATP-1B3 transporter.

Because it is a CYP3A4 substrate, its activity is increased by grapefruit juice and other CYP3A4 inducers, while it is decreased by CYP3A4 inhibitors like St. John's wort. The action of potent CYP3A4 pharmacological inducers can be tripled. Pomegranates and starfruit, according to patients, may potentially cause problems.

Food shouldn't be consumed two hours before or one hour after because doing so unexpectedly doubles its bioavailability.

2.8 Brand names (Nilotinib) - Tasigna capsule

3 Experimental work

3.1 List of Instruments and chemicals

Table 2 List of Chemicals

Sr No	Chemicals /Solvent/Reagent	Grade	Make	Characterization
1	Methanol	HPLC	Research lab	As HPLC grade no further characterization performed
2	HPLC Water	HPLC	Research lab	As HPLC grade no further characterization performed

3.2 All other chemical were used of analytical grade

Table 3 List of Apparatus

Name	**Make By**
HPLC Model	Younglin (S.K) Gradient System UV Detector
UV-Vis Spectrophotometer	Shimadzu-UV 1800
Electronic Balance	Citizen CY220
Sonicator	Chief Scientific Industry(3.5l)
Glassware's	Borosil
Filters	0.45µ Nylon, Teflon

3.3 Identification of drug

3.3.1 Determination of Analytical wavelength

3.3.2 Preparation of Standard Stock solution of Nilotinib

An Accurately weighed quantity of about 10mg of Nilotinib was taken in 100 ml volumetric flask dissolved in sufficient quantity mobile phase (methanol : Water ,60:40), then sonicated for 15 min and diluted to 100ml with the same solvent so as to get the concentration of 100µg/ml. The resultant solution was scanned using UV visible spectrophotometer in the range of 200-400nm. The Standard λmax of Nilotinib is 262 nm

3.3.3 Selection of Analytical wavelength

The wavelength selected was 260 nm.

3.3.4 Melting point

The melting point of Nilotinib was determined by capillary method using digital melting point apparatus and found to be 235° C.(Standard-230 ° C-240 ° C)

3.4 HPLC method development

3.4.1 Selection of mobile phase

The aim is to find the correct concentration of the mobile phase. The mobile phase and its strength is a measure ability to pull analyte from the column ,The standards and sample solutions of Nilotinib was prepared in mobile phase. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram. The mobile phase that was found to be most suitable Methanol :Water (0.1 % acetic acid) 80: 20 v/v was selected for the evaluation of the chromatogram of drug.

3.4.2 Selection of column

The Column selection is important step in method development by HPLCsystem. Generally, C-18 Column is selected for high performance liquid chromatography. So, here C-18 {4.6 x 250 mm (5µm)} column with UV detector was used for the study

3.4.3 Preparation of stock solution

Standard stock solution was prepared by dissolving accurately weighed 10 mg of Nilotinib in 10 ml of mobile phase that gives concentration of 1000 µg/ml, and sonicated for three cycles each of 10 min.

3.4.4 Preparation of Stock solution from dosage form (capsule)

Twenty capsules (04g) were weighted; average weight was determined and powdered. Powder equivalent to 10mg (10 x 200/200= 10mg) was transferred to 10ml of mobile phase i.e 1000 µg/ml. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min. From this 20 µg/ml is taken for assay.

3.4.5 Preparation of Acidic water (0.1% acetic acid)

0.1g of acetic acid is transferred to 100 ml volumetric flask and dissolved in sufficient quantity of water and volume is made up with water. The resulting water was sonicated for three cycles each of 10 min.

3.4.6 System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. System Suitability Requirements are The relative standard deviation for the peak area response of standard preparation is not more than 2.0 %.

The column efficiency for Nilotinib peak should not less than 2000 theoreticalplates.
The tailing factor (asymmetry factor) for Nilotinib peak, should not more than 2.0.

3.5 Method validation of HPLC

3.5.1 Linearity

From standard stock solution, aliquots of 0.05, 0.1, 0.15, 0.2,and 0.25ml were taken in 10 ml volumetric flasks and diluted up to the mark with Mobile phase such that to obtained concentration of 05µg/ml , 10µg/ml , 15µg/ml , 20µg/ml , 25µg/ml , of Nilotinib respectively. Volume of 20 µL of each sample was injected with the help of Hamilton syringe. All measurements were repeated two times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration, Results were recorded for equation of line; correlation coefficient and intercept were determined.

$$Y= mX+c$$

Equation 1 Linearity

Where, Y- area

X- Concentration

m- Slope of graph

c- Intercept

3.5.2 Precision

Precision is the measure of how close the data values are to each other for a numberof measurements under the same analytical conditions. It was verified by repeatabilityand intermediate precision studies. Intra-day precision was studied by analyzing 10, 15, 20µg/ml of Nilotinib for three times on the same day. Inter day precision was checked by analyzing the same concentration for three different days over a period of weak Results were recorded to calculate mean, SD, %RSD.

3.5.3 % Accuracy

The accuracy of the method was studied by recovery study using standard addition method at 80%, 100% and 120% level. , 10µg/ml capsule solution is taken, To the 0.1ml of the capsule solution 0.08ml, 01ml, and 0.12 ml standard solution were added separately it is 8µg/ml, 10µg/ml, 12µg/ml respectively and analyzed by the proposed HPLC method.

3.5.4 Robustness

Robustness of the method was studied by making deliberate changes in few parameters viz; flow rate, wavelength and mobile phase combination. The effects on the results were studied by injecting 20 µg/ml of Nilotinib ; one factor was changed attime to estimate the effect.

Table 4 Robustness variations Table

Condition	Normal	Variation 1	Variation 2
Flow rate	0.8ml/min	0.7ml/min	0.9 ml/min
Wavelength	260nm	261nm	259nm
Mobile phase	80:20	79:21	81:19

3.5.5 LOD and LOQ

LOD and LOQ were determined using following formulas

$$LOD = 3.3*I/ (SD)$$
$$LOQ = 10*I/ (SD)$$

Equation 2 LOD and LOQ

Where,

I = Intercept of the graph,

SD = Standard deviation

3.5.6 Repeatability

It is measured by 5 injections of a sample of 15 µg/ml of Nilotinib that indicates theperformance of the HPLC instrument under chromatographic conditions

3.5.7 Analysis of capsules (Marketed formulation)

For the analysis of capsule Twenty capsules (04g) were weighted; average weight was determined and powdered. Powder equivalent to 10mg (10 x 200/200= 10mg) was transferred to 10 ml of mobile phase ,it gives concentration of 1000 µg/ml.The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min. The aliquots was subjected to proposed method and amount of Nilotinib was determined

3.5.8 Brand Name- Tasigna (Novartis Ltd)

4 Result and discussion (validation)

4.1 Characterization of drug

4.1.1 Melting point

The melting point of Nilotinib was determined by capillary method using digitalmelting point apparatus and found to be 235º C.(Standard-230 º C-240 º C)

4.1.2 Determination of wavelength:

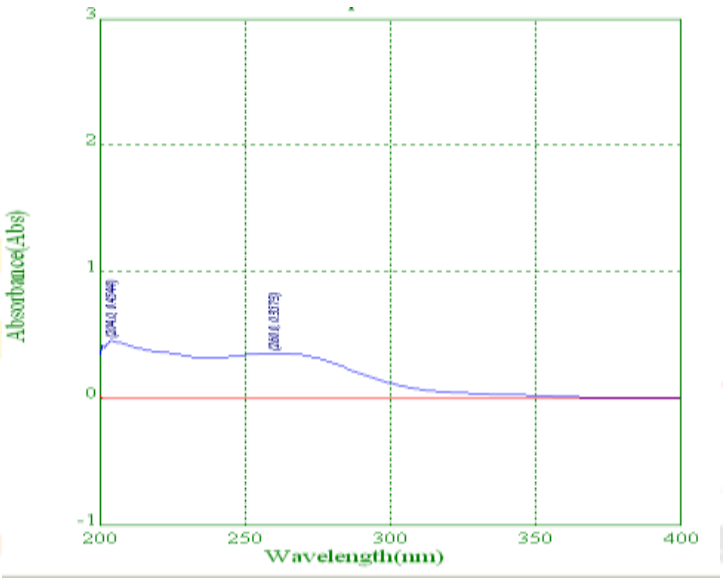


Figure 1 wavelength spectra

Table 5 UV Analysis for Detection of Wavelength

Sr.No.	Wavelength (nm)	Absorbance
1	204nm	0.4544
2	260nm	0.3573

Discussion:

10µg/ml solution of Nilotinib was prepared in the mixture of Methanol : water(60: 40) was subjected to UV analysis in qualitative mode to determine the absorption maxima (λ max). 204 and 260 nm and absorbance was recorded as 0.4544 and 0.3573 respectively. The wavelength of 260 was selected for quantitative determination of Nilotinib as given in further sections. The wavelength spectra is shown in figure 1 and the data for detection of wavelength is shown in table 5

4.2 HPLC method development

4.2.1 Selection of mobile phase

The preferred mobile phase methanol (80) : water (20) (0.1% Acetic acid) ableto analyze the Nilotinib at selected chromatographic condition. This mobile phase compositions provided superior resolution and most favorable retention time with appropriate tailing factor.

4.2.2 Selection of column

HPLC system with C18 (4.6 x 250 mm, 5 µm) analytical column and UV visible detector was selected for method development. C_{18} columns afford shorter retention and equilibration times. This column is recommended for Nilotinib method development.

4.2.3 Method development Trial

From the literature review the various mobile phase are used to develop a easy, economical and fast method development. Here some of the chromatograms formethod development trials are shown in Figure 2 and Figure 3. The final chromatogram for developed method in Methanol (80) : water (20) (0.1% acetic acid)is shown in Figure 4

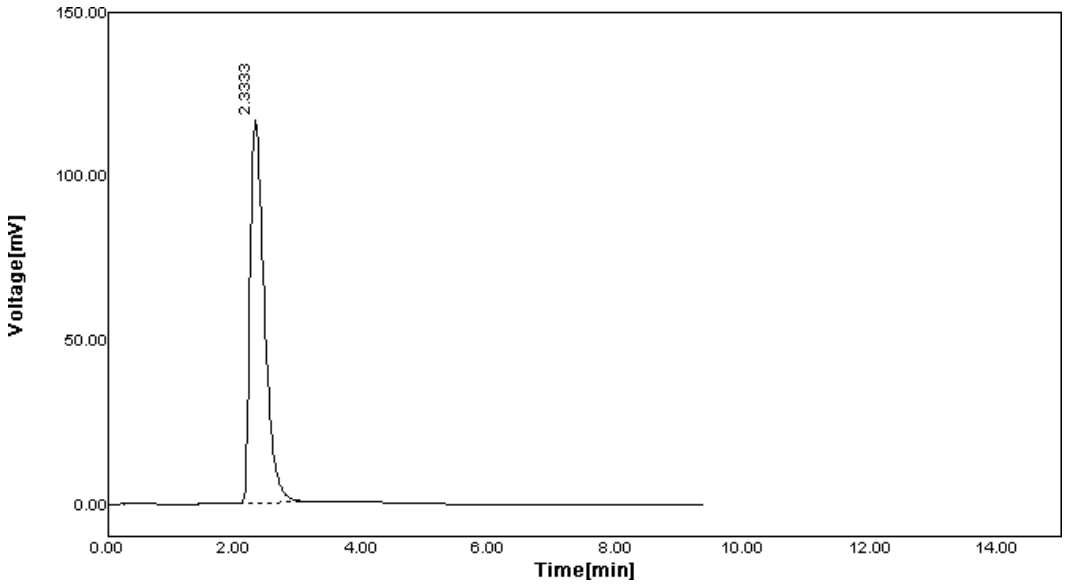


Figure 2 Trial 1 : methanol(90): (10)water

RT[min]	Area[]	Area%	TP	TF	Resolution
2.3333	18.230131	100.00	523.6	1.6265	0.0000

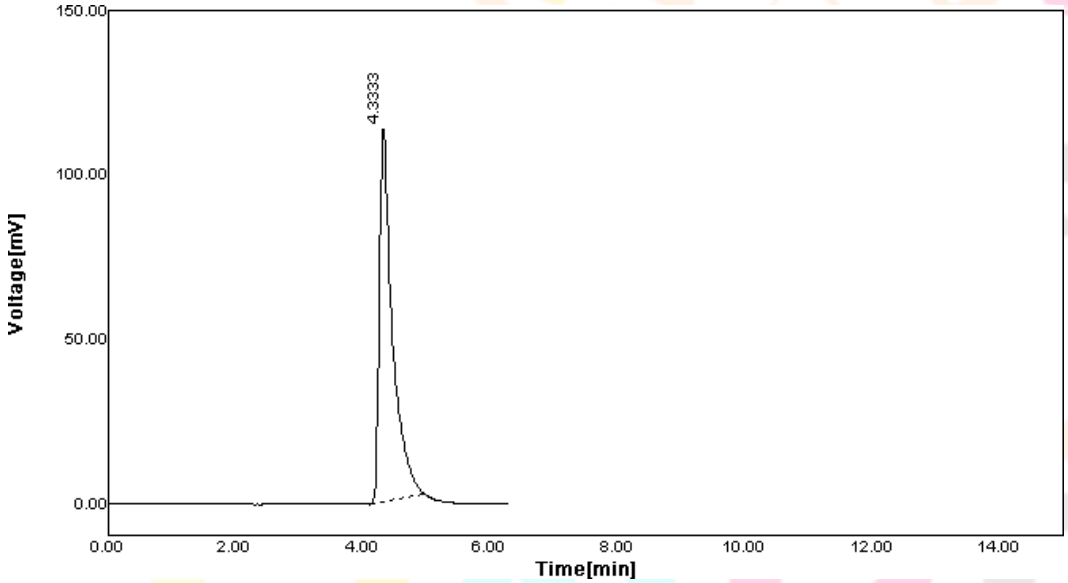


Figure 3 Trial 2 : methanol(80) : water(20)

RT[min]	Area[]	Area%	TP	TF	Resolution
4.3333	16.332426	100.00	2459.1	2.2028	0.0000

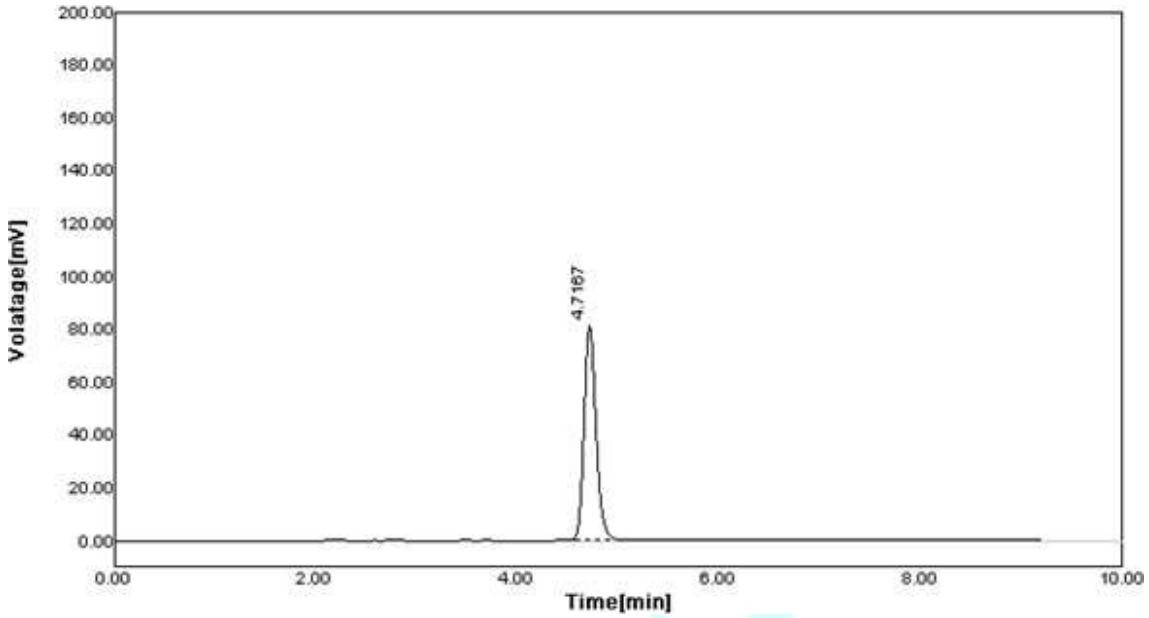


Figure 4 Standard chromatogram methanol(80) :Water 20 (0.1 % acetic acid)

RT[min]	Area	Area%	TP	TF	Resolution
4.7167	67.29604	100.00	5482.8	1.2857	0.0000

4.2.4 System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing; results are shown in Table 6

Table 6 Data Showing System Suitability Test

System suitability parameters	Proposed method
Retention time (tR)	4.7
Theoretical plate (N)	5482.8
Tailing factor (T)	1.2857
Resolution	0.0000

4.2.5 Chromatographic condition

Table 7 Chromatographic condition

HPLC	Younglin (S.K) Gradient System UV Detector
Detecter & pump No.	UV 730 D & SP930 D
Software	Autochro -3000
Column	4.6 x 250 mm
Particle size packing	5 m
Stationary phase	C_{18}
Mobile Phase	Methanol (80) : Water(20) (0.1 % Acetic Acid)
Wavelength	260 nm
Flow rate	0.8 ml/min
Injection volume	20 □l

Column temp	Ambient
Run time	4.71min

5 HPLC Method validation

5.1 Linearity

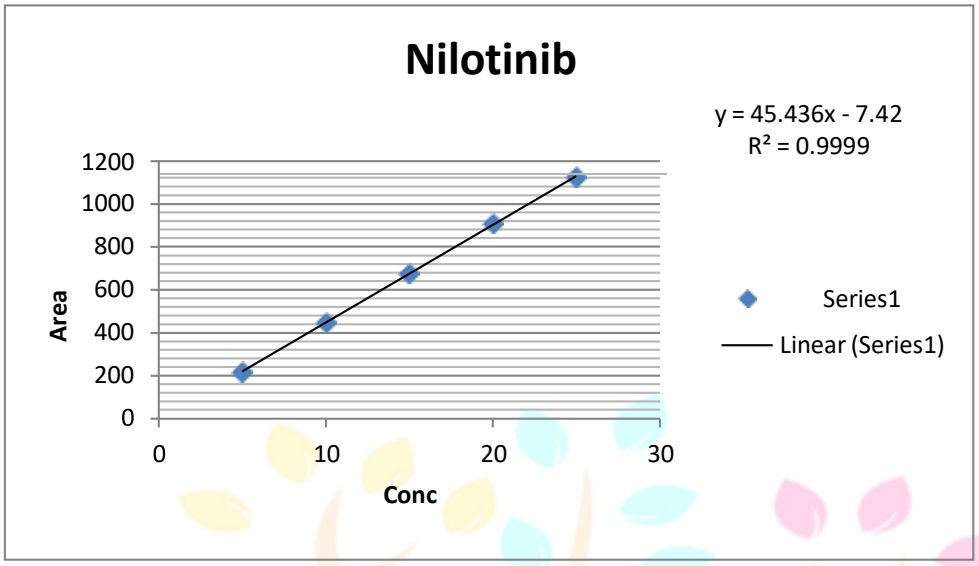


Figure 5 Linearity Graph by HPLC of Nilotinib Conc.Vs area

Table 8 Linearity data

Sr No.	Conc	Mean Peak area	SD	%RSD
1	5	218.865	1.28	0.58
2	10	446.03	1.63	0.36
3	15	674.405	2.04	0.30
4	20	907.115	1.32	0.15
5	25	1124.23	1.97	0.17
		Avrg SD	1.65	

Table 9 Linearity parameter

Equation	Y=45.43X+7.4
Slope	45.43
Intercept	7.4
Regression	0.999

Discussion:

The linearity of an analytical procedure is its ability to elicit test results that are proportional to the concentration of analyte in the sample. From stock standard solution, aliquots of 0.05 , 0.1, 0.15, 0.2, 0.25ml were taken in 10 ml volumetric flasks and diluted up to the mark with Mobile phase such that to obtained concentration of Nilotinib in the range 5-25µg/ml. All measurements were repeated

Two times for each concentration and calibration curve was constructed by plotting thepeak area *versus* the drug concentration. The data obtained for linearity study for Nilotinib are shown in Table 8 and parameter in table 9 . The calibration curve are plotted by using concentration verses area for Nilotinib in Figure 5

5.2 Precision

Precision is the measure of how close the data values are to each other for a numberof measurements under the same analytical conditions. It was verified by repeatabilityand intermediate precision studies. Intra-day precision was studied by analyzing 10, 15 and 20µg/ml of Nilotinib for three times on the same day. Inter day precision was checked analyzing the same concentration for three

different days over a period of weak. The results are shown in table 10. And 11. The chromatogram of intermediate precision are shown in Figure 6, Figure 7 and Figure 8 respectively.

Table 10 Intraday precision data

Sr No.	Conc	Mean peak area	SD	%RSD
1	10	445.76	1.24	0.28
2	15	679.29	0.89	0.13
3	20	907.00	1.49	0.16

Table 11 Interaday precision data

Sr No.	Conc	Mean Peak area	SD	%RSD
1	10	445.74	4.93	1.11
2	15	675.54	1.25	0.19
3	20	907.23	3.67	0.40

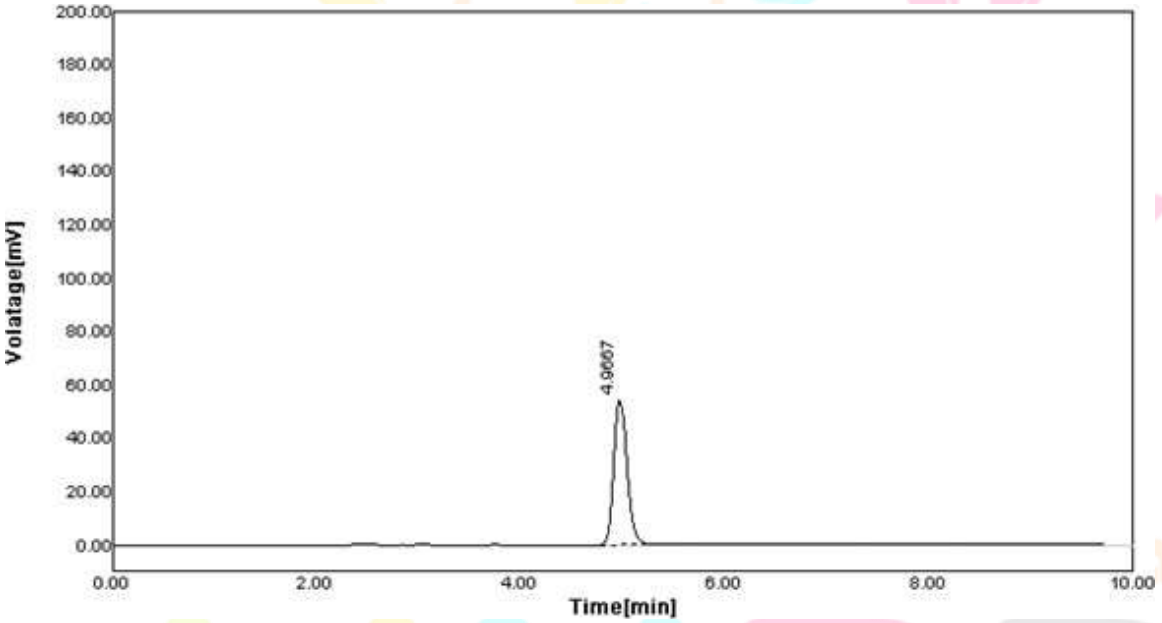


Figure 6 Chromatogram for Precision 1

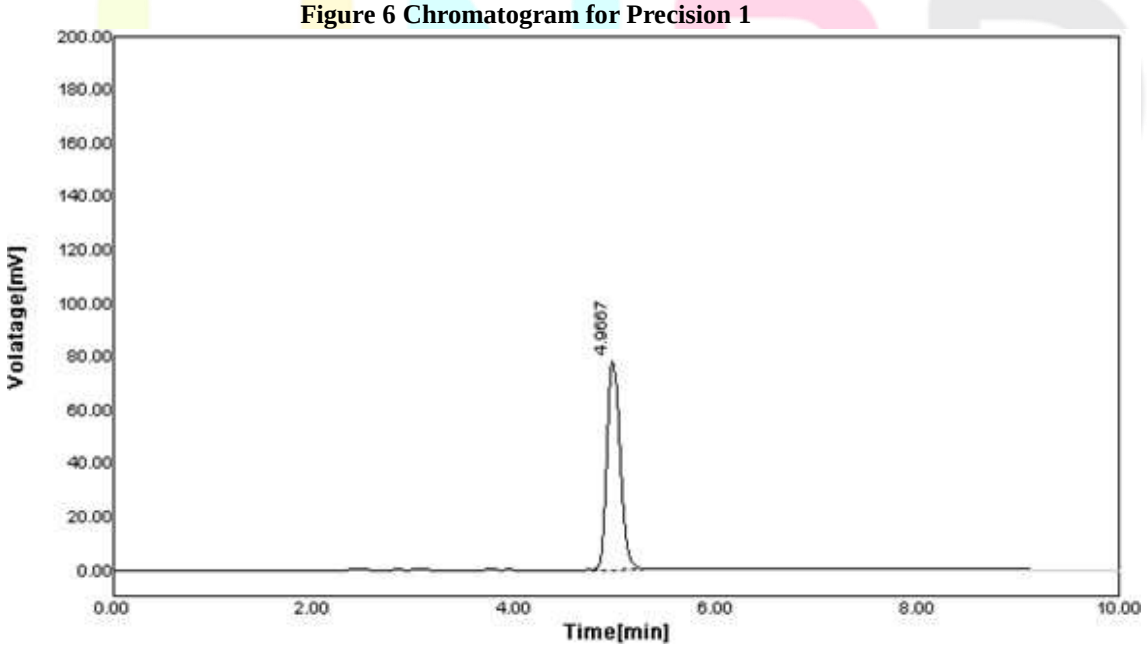


Figure 7 Chromatogram for Precision 2

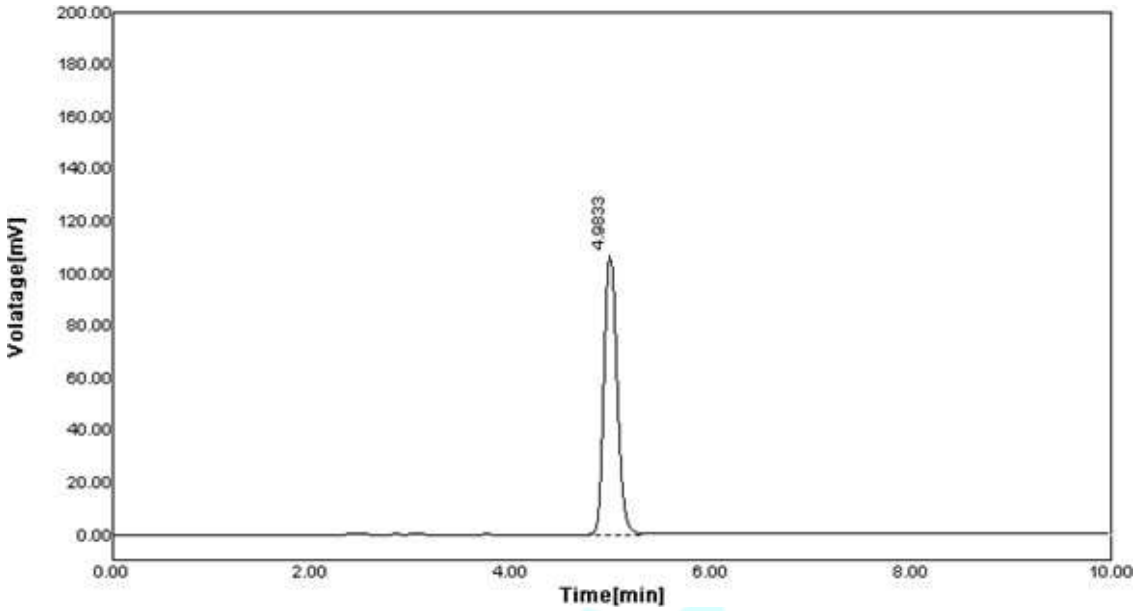


Figure 8 Chromatogram for Precision 3

5.3 Accuracy

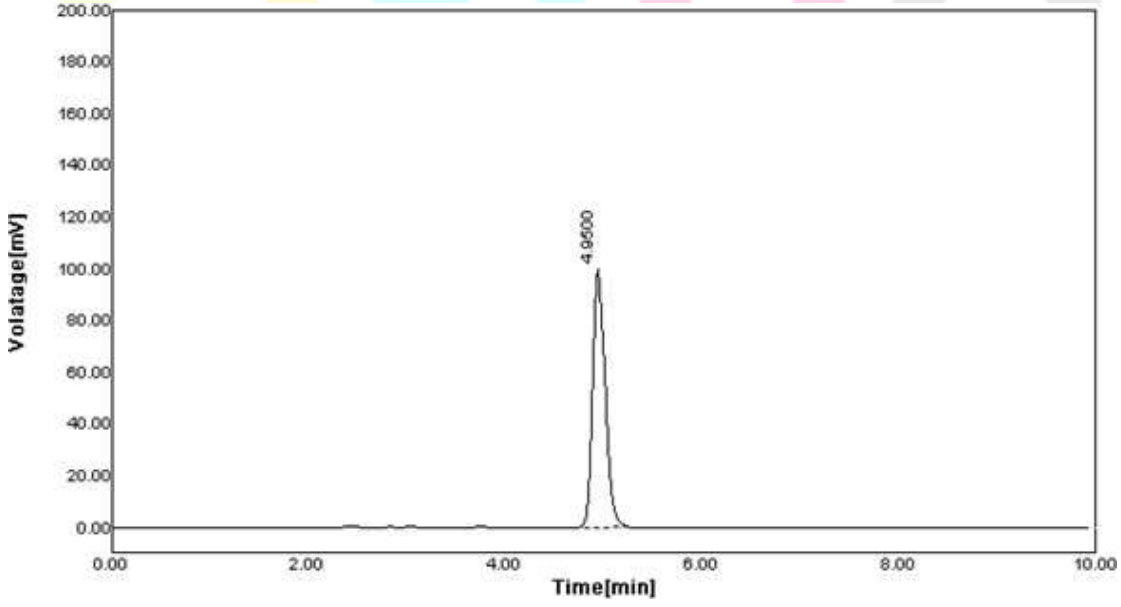
Table 12 Accuracy data

Recovery level %	Concentration (µg/ml)	Amnt Added	Amount recovered ±SD (µg/ml)	Recovery %	% RSD
80	20	8	7.81	98.28	0.65
100	20	10	20.58	99.60	1.14
120	20	12	11.81	101.58	0.73

Discussion

The accuracy of the method was studied by recovery study using standard addition method at 80%, 100% and 120% level. To the 0.1ml of the Capsule solution 0.08ml, 01ml, and 0.12 ml standard solution were added separately it is 8µg/ml, 10µg/ml, 12µg/ml respectively and analyzed by the proposed HPLC method. The results for recovery study are shown in Table 12. The chromatograms obtained at standard 80 % addition, 100 % addition, 120 % addition are shown in Figure 9 , Figure 1 0 andFigure 11 respectively

Figure 9 % Recovery Chromatogram 80%



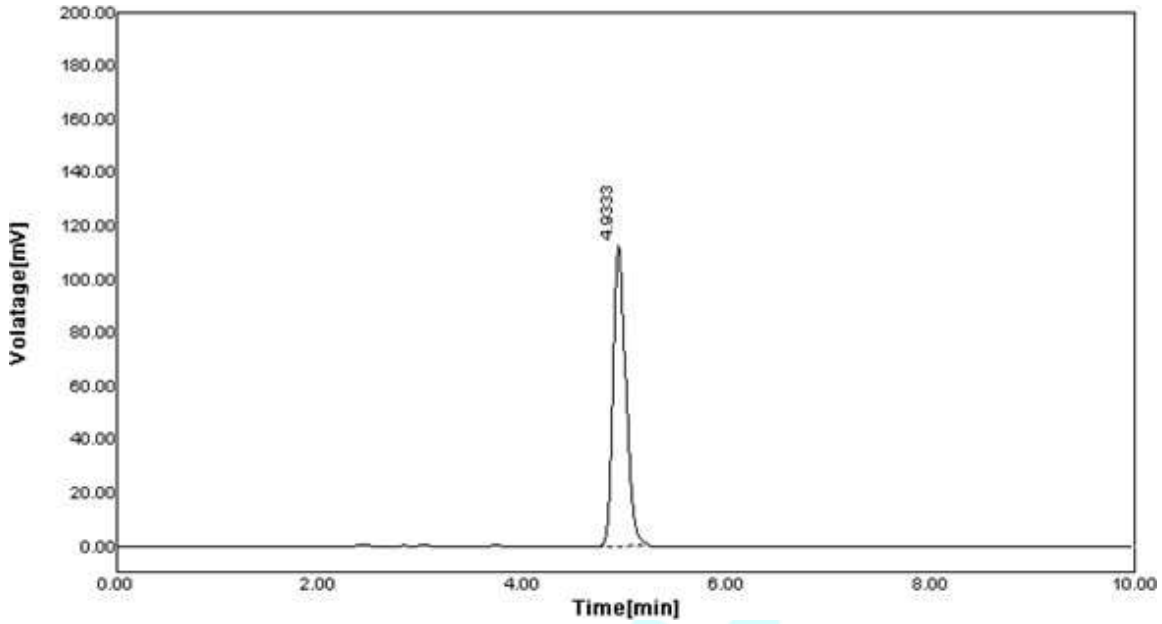


Figure 10 % Recovery chromatogram 100 %

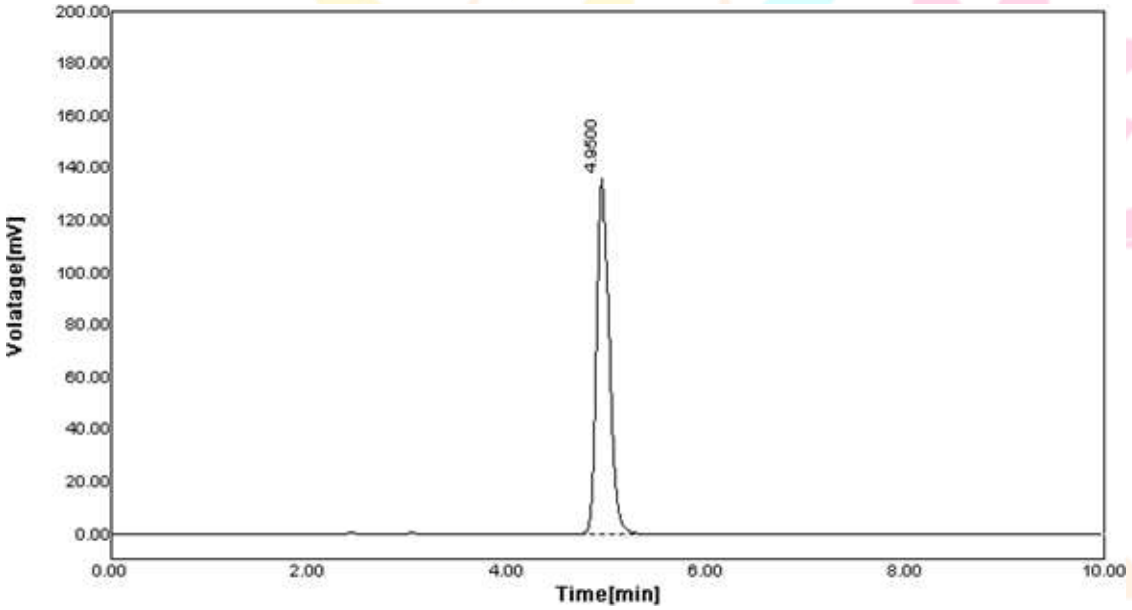


Figure 11 % Recovery chromatogram 120 %

5.4 Robustness

Table 13 Data for Robustness study

Parameter	% RSD	
Flow rate (±0.1 ml) (Original-0.8ml/min)	0.7ml/min	0.20
	0.9ml/min	0.13
Wavelength (±1 nm) (Original-260 nm	259nm	0.05
	261nm	0.17
Mobile phase combination (±1) (Original-Methanol : water (0.15Acetic acid (80:20 v/v)	79:21	0.19
	81:19	0.25

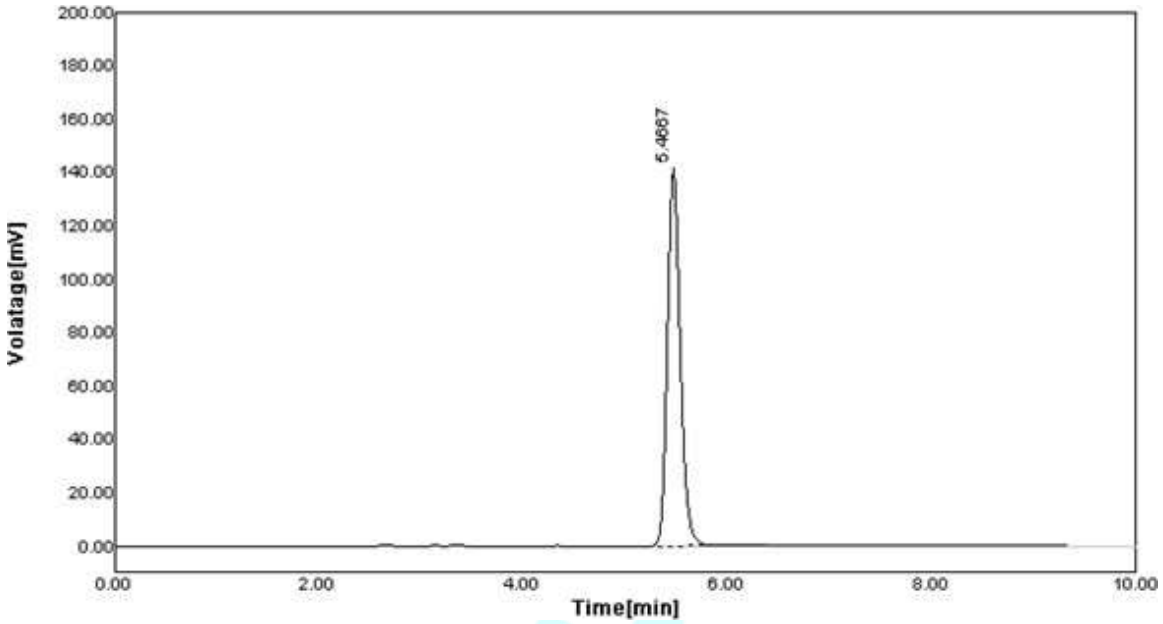


Figure 12 Chromatogram Robustness flow change 0.7ml/min

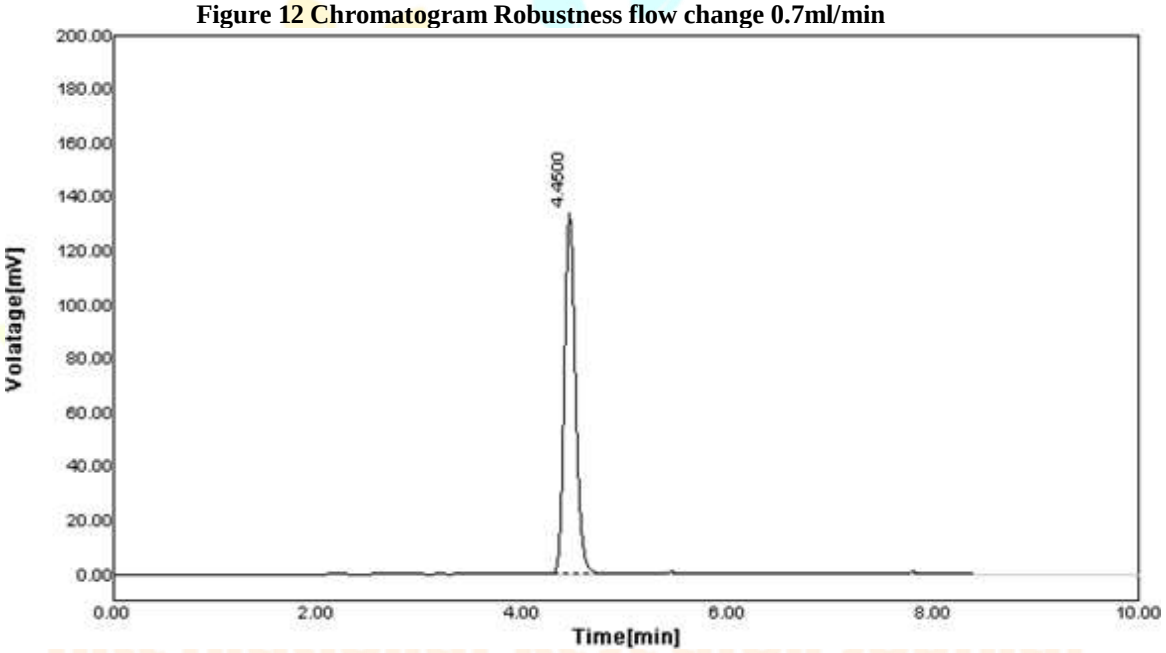


Figure 13 Chromatogram Robustness flow change 0.9 ml/min

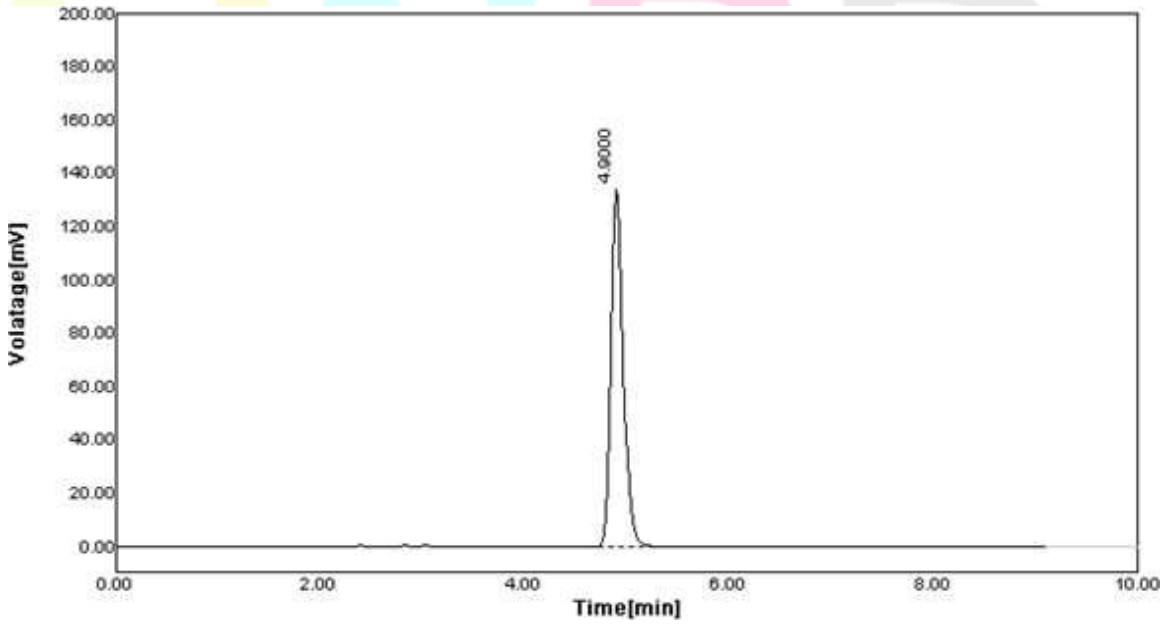


Figure 14 Chromatogram Robustness wavelength change 261nm

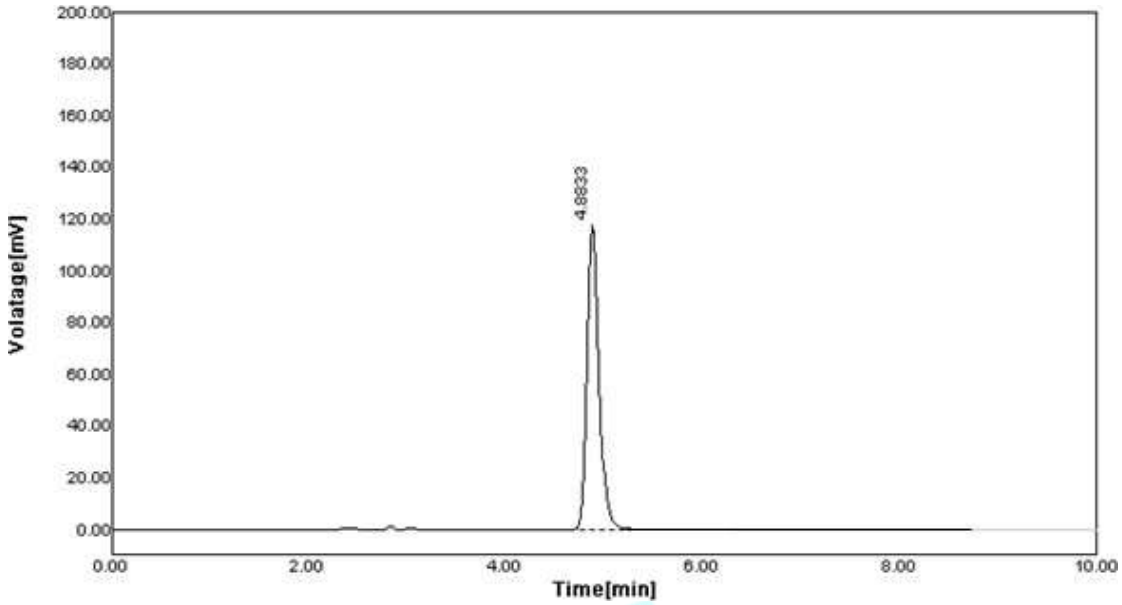


Figure 15 Chromatogram Robustness wavelength change 259 nm

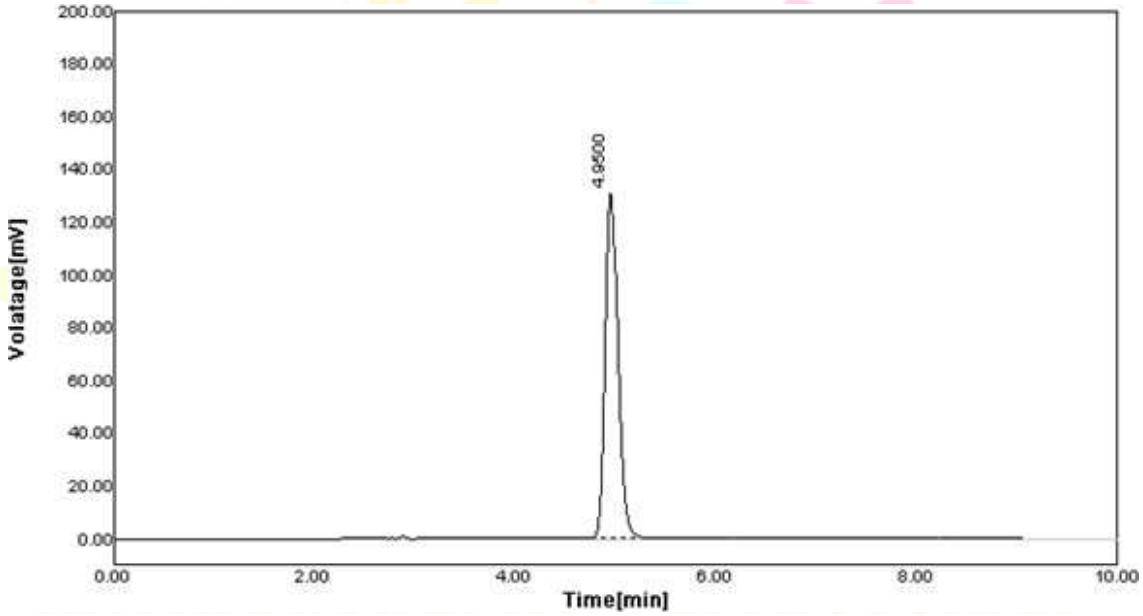


Figure 16 Chromatogram Robustness mob phase conc change 79:21

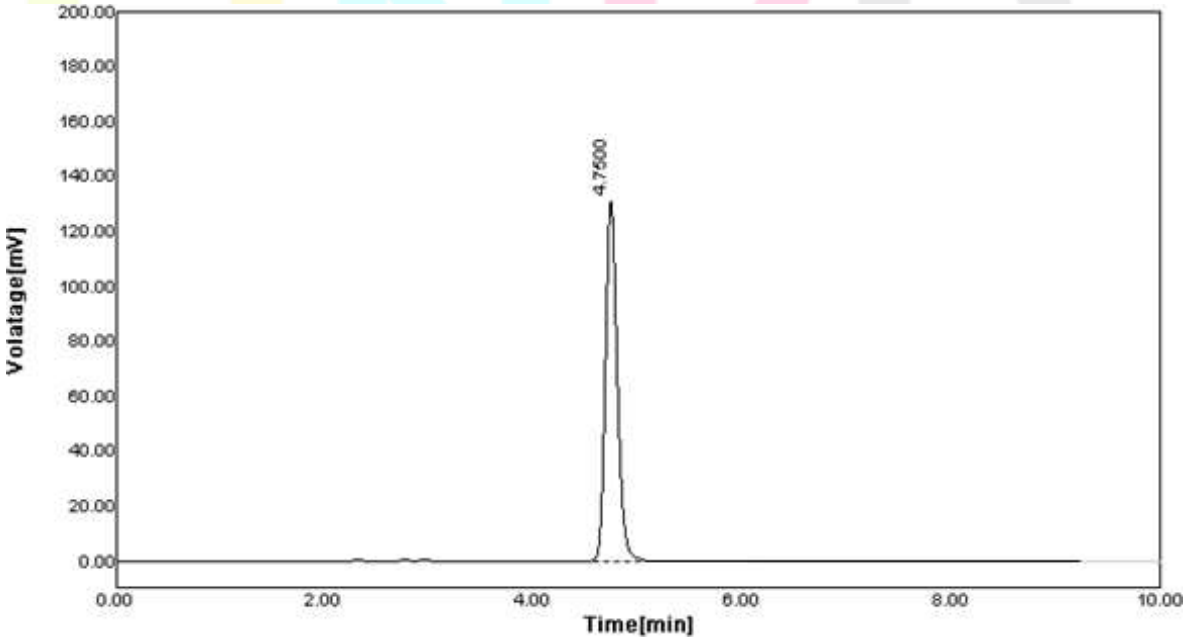


Figure 17 Chromatogram Robustness mob phase conc change 81:19

Discussion:

Robustness of the method was studied by making deliberate changes in few parameters viz; flow rate, wavelength and mobile phase combination. The effects on the results were studied by injecting 20µg/ml of Nilotinib; Data for robustness study is shown in table 13 and figures are shown in figure 12-figure 17

5.5 LOD and LOQ

Table 14 LOD and LOQ data

LOD(g/ml)	LOQ(g/ml)
0.1198	0.3631

Discussion:

The limits of detection (LOD) and quantitation (LOQ) were determined separately, The LOD were found to be 0.1198 ug/ml .The LOQ were found to be 0.3631 ug/ml ,LOQ and LOD data are shown in table 14.

5.6 Repeatability

Table 15 Data Showing Results of Repeatability Studies

Sr No.	Conc	Mean	SD	% RSD
1	15	672.95	2.91	0.43

Discussion

It is measured by 5 injections of a sample of 15 µg/ml of Nilotinib that indicates the performance of the HPLC instrument under chromatographic conditions,data for repeatability study are shown in table 15 and chromatogram are shown in figure 18-22

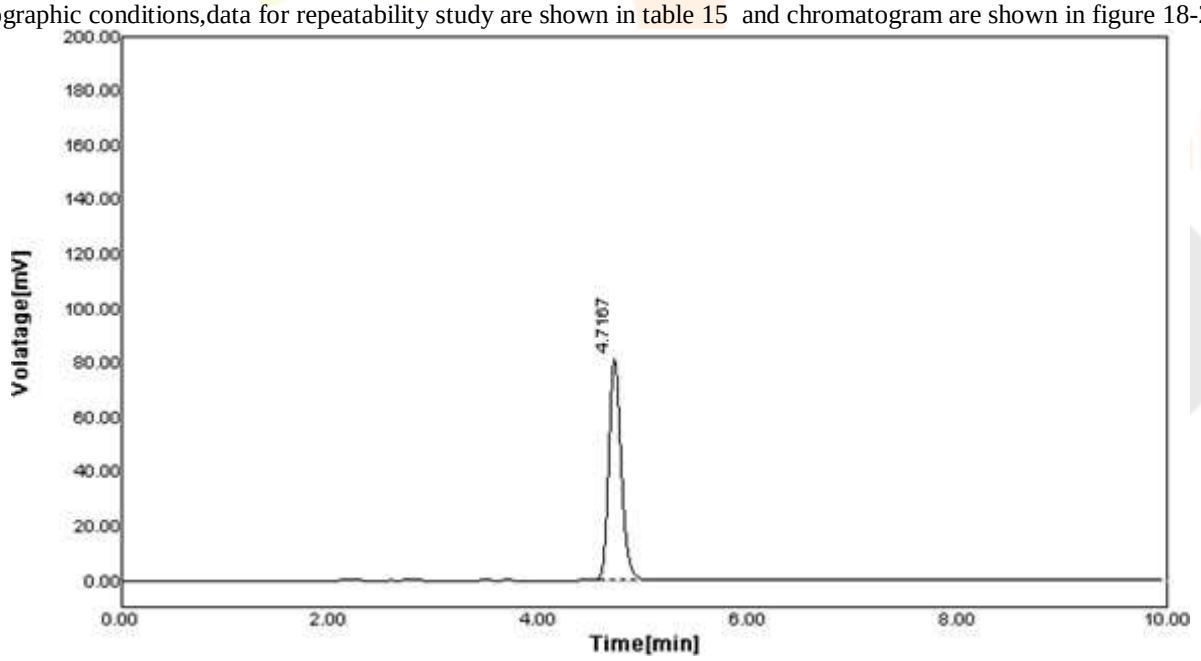


Figure 18 I chromatogram for Repeatability

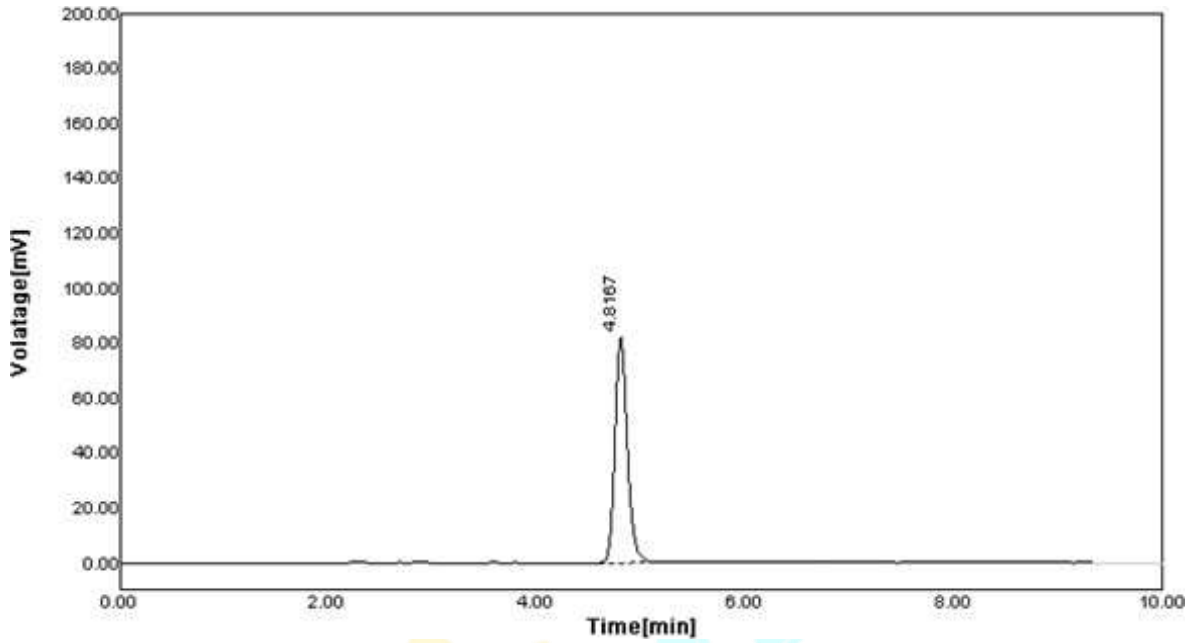


Figure 19 II chromatogram for Repeatability

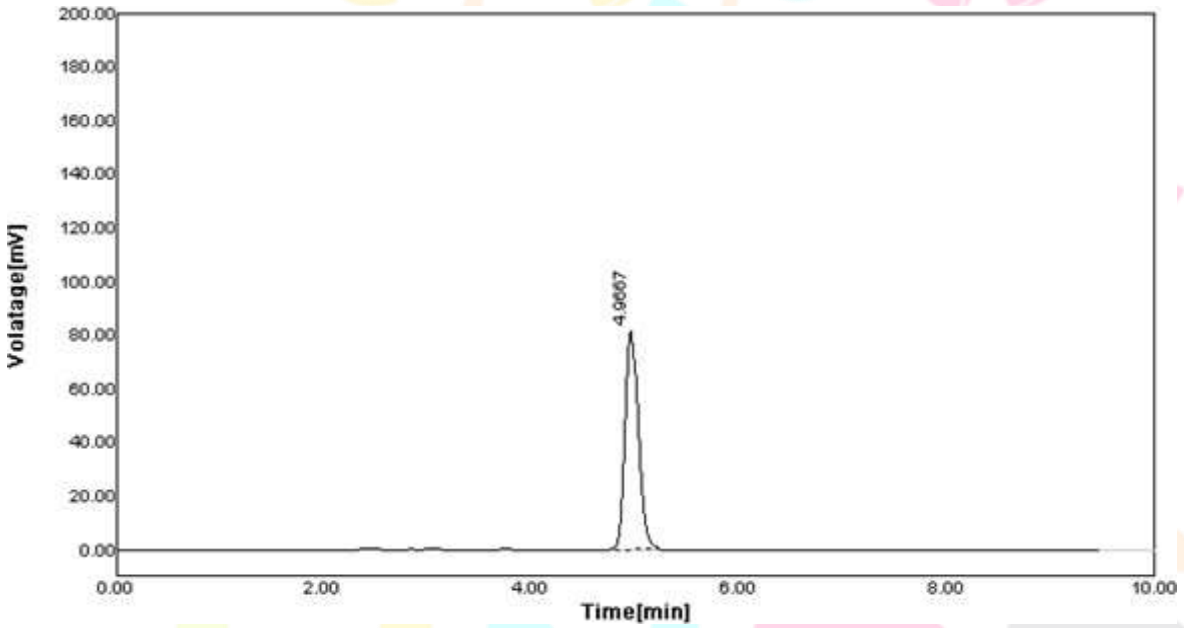


Figure 20 III chromatogram for Repeatability

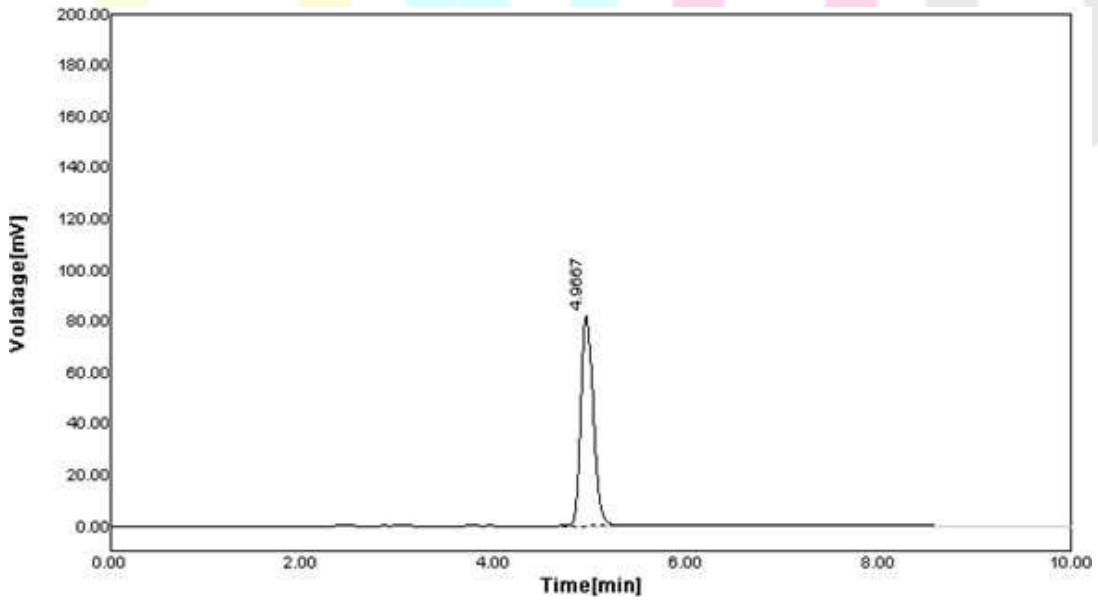


Figure 21 IV chromatogram for Repeatability

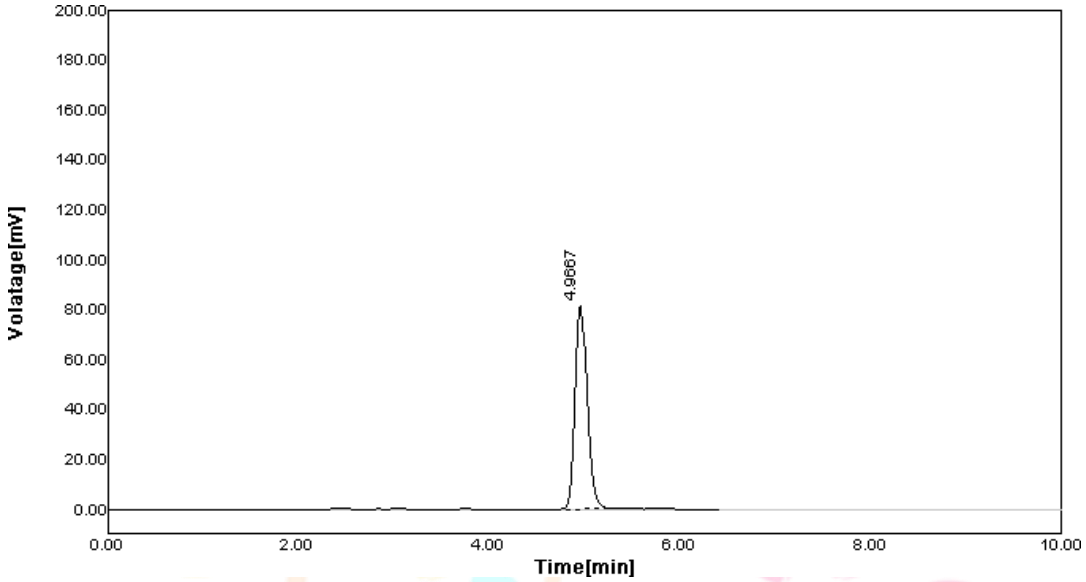


Figure 22 V chromatogram for Repeatability

5.7Analysis of capsules (Marketed formulation)

Table 16 Analysis of capsuie

Sr. no	Component	Label claim(mg)	Amount found (mg) ±SD	% Label claim	% RSD
1	Nilotinib	200	14.53	96.83	0.28

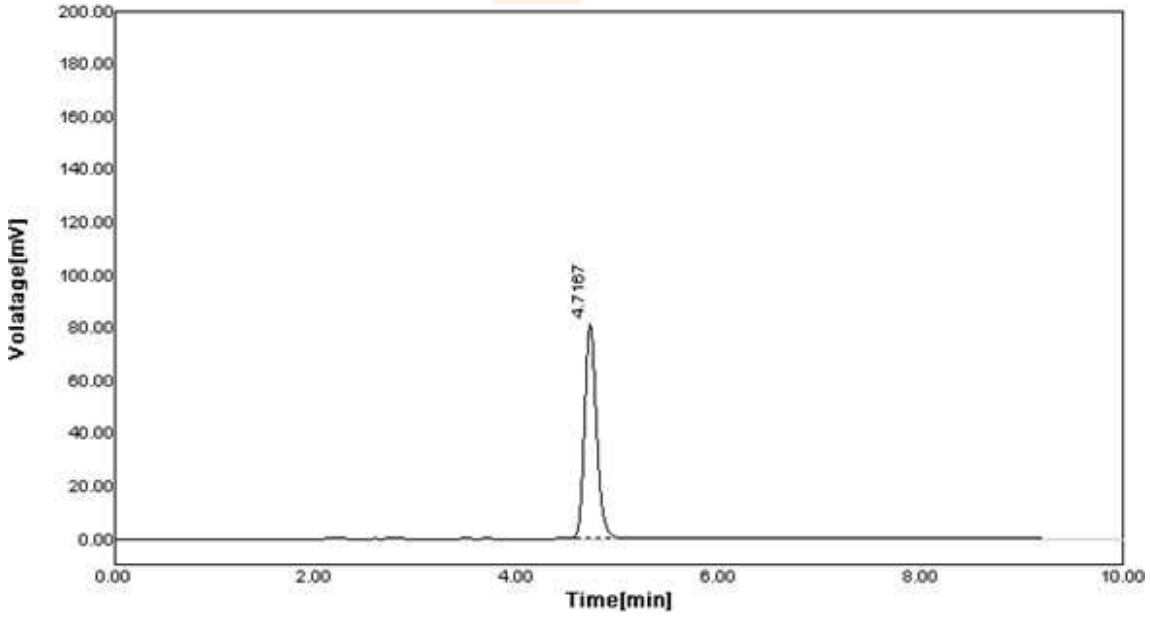


Figure 23 Chromatogram for analysis of capsule

Discussion

For the analysis of capsule Twenty capsules (200mg) were weighted; average weight was determined and powdered. Powder equivalent to 10mg (10 x 200/200= 10mg) was transferred to 10 ml of mobile phase ,it gives concentration of 1000 µg/ml. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min.The solution was further diluted to get concentration of 20 µg/ml of Nilotinib. The aliquots were subjected to proposed method and amount of Nilotinib was determined. The chromatogram for capsules assay is shown in Figure 23 and result are mentioned in Table 16

5.8 Method validation summary

Table 17 Method validation summary

Parameter		Result
Specificity		Specific
Linearity and range		5-25µg/ml
Precision %RSD	**Interday**	0.28-0.16
	Intraday	1.11-0.40
Accuracy Amount recovered ±SD (µg/ml)	**80%**	7.81
	100%	20.58
	120%	11.81
Robustness		The system suitability parameter were found well within the acceptance criteria as per system suitability .
LOD(g/ml)		0.1198
LOQ(g/ml)		0.3631
Repetability %RSD		0.43
% Assay		0.28

Discussion

The proposed estimated method was found to be simple, precise, accurate and rapid for the determination of Nilotinib from Capsule forms, the mobile phase is simple to prepare and economical. The sample recoveries in all the formulations were in good agreement with their respective label claim and their suggestive not interference of formulation excipients in the estimation. Hence this method can be conveniently adopted for routine analysis of Nilotinib in the pharmaceutical dosage forms.

High Performance Liquid Chromatographic Method

Attempts were made to Develop HPLC method for estimation of Nilotinib. HPLC method was developed and validated as per ICH guidelines by using a mobile phase consisting mixture of Methanol:Water (80:20), at the flow rate of 0.8ml/min. A C_{18}column was used as stationary phase. The eluent were detected at 260 nm. Statistical analysis of the method was done by using way analysis of variance .The method was found to be simple, accuracy, precise, economical and reproducible. So the propose method can be used for the routine quality control analysis of Nilotinib in bulk drug & dosage form.

REFERENCE

1. C. Christian ; Analytical Chemistry, 6th Edn, (2008) 822-828
2. M.Z .Ahmad ; Mohammad Ali ; Textbook of Pharmaceutical Drug Analysis, 1stEdn. 2009. 2287
3. P. Parimoo ; Pharmaceutical Analysis,.2005.365-370
4. K.A. Connors ; A textbook of Pharmaceutical Analysis, 3rd ed. John Wiley andsons, (1999) 277-288
5. A. V .Kasture ; S. G. Wadodkar,; K. R. Mahadik,; H .N. More ; PharmaceuticalAnalysis: Instrumental methods II, 11th Edn, (2004) 7
6. G. R. Chatwal ; A. K. Sham ; Instrumental methods of Chemical Analysis, 5thEdn. (2008,)2.599-2.5605
7. H. H. Willard, ; L. L Merrit ; J. A Dean ; F. A. Settle; Instrumental method ofanalysis, 7th Edn. 1995, 1-3
8. Scott P.W.; Principles and practice of chromatography., 3rd ed., Chrome bookseries, Victoria, **2007.** pp 05-30.

9. Sudhakar Babu Kundra;Venkatramanna medreddy; Narayana reddy papadasu; Latha Jonnalagad; a validated stability indicative UPLC method for the Nilotinib hydrochloride for determination of process related and degredation impurities
,Journal Of Chromatographic Science,july 2013
10. Stephane Bouchet ; Simultaneous determination of nine tyrosine kinase inhibitorsby 96-well solid-phase extraction and ultra performance LC/MS-MS,Clinica Chimica Acta,vol 412,12 may 2011,pp-1060-1067
11. Silvia Di Francia ; New HPLC–MS method for the simultaneous quantificationof the antileukemia drugs imatinib, dasatinib, and nilotinib in human plasma,Journal of chromatography, vol 877,june 2009,pp 1721-1726
12. Nilotinib drugbank DB04868
13. Nilotinib/Wikipedia /the free encyclopedia
14. Nilotinib//Medline plus.drug information
15. International Conference On Harmonization (ICH), (1997), Q2b: Validation OfAnalytical Procedure: Methodology, USFDA Federal Register, (Vol. 62), 27463
16. International Conference On Harmonization (ICH), (1995), Q2a: Text OnValidation Of Analytical Procedure,USFDA Federal Register, (Vol 60), 11260
17. B.K. Sharma ; Instrumental Methods of Chemical Analysis, 25th Edn. Meerut,(1983) S82
18. D. A.Skoog ; F.J. Holler ; S.R. Crouch ; Principle of Instrumental Analysis, 6thEdn. (2007). 893-900
19. A.H.Beckett ; J.B.tenlake ; Practical Pharmaceutical chemistry, Part II, 4th Edn.(2005) 384
20. www.hplc.Sciencedirect.com
21. www.hplc.Wikipedia.com
22. Raymond P ; Scott ; 'Liquid chromatography for the analyst ,chromatographicscience series ',1st ed, Marcel Dekker,inc 1994,1-30
23. Synder L.R. and Kirkland J.J., "Practical HPLC Method Development"2nd edition,John Wiley and Sons, Inc.,1997,2-45.
24. S. Ahuja ; H. Rasmussen ; Analytical method development, Validation: HPLCmethod development for Pharmaceuticals, vol.8. 95-102
25. S. Ahuja ; S. Scypinski ; Degradation study: Handbook of Modern Pharmaceutical Analysis, Separation and Technology, vol.3. 55-60
26. S. Chandran ; R. Singh ; "Comparison of Various International Guidelines forAnalytical Method Validation." Pharmazie. (2007) 48
27. FDA "Analytical Procedures and Methods Validation Chemistry, Manufacturingand Controls Documentation; Availability, " Federal Register (2000)
28. US FDA, General principles of validation, Rockville, MD, Center for Drug Evaluation and Research (CDER), May 1987.2-20
29. US FDA, Guidelines For Submitting Samples And Analytical Data For Method Validation Rockville, MD, Center for Drugs and Biologics Department of Health and Human Services, Feb. 1987. 26-34
30. Myneni Harika ; G.S kumar ; Development and validation method for the determination if Nilotinib by RP-HPLC in bulk and pharmaceutical dosage form ,International Research Journal Of Pharmacy ,ISSN No 2230-8407,2012 (3) 17/12/12
31. L Satyanarayana ; Prof. S V Naidu ; Prof. M Narasimha Rao,; Reddy Suma Latha; The estimation of Nilotinib in capsule dosage form by RP-HPLC , Asian Pharma Tech 2011 (vol I), 19 june 2014 ,pp 82-84
32. E.M.Yilmaz ; Z. Aydogmus,; H.Y .Aboul-Enein ; Determination of Nilotinib in spiked plasma,urine and capsule by High Performance Liquid Chromatography with flurimetric detection , Acta Chromatographica, DOI 10.1556/1326 ,2016
33. G.Sowjanya ; M.Mathrusri ; Annapurna ; A Venkata Sriram; Development and validation of stability indicating RP-HPLC method for determination ofNilotinib, Indo American Journal of Pharmaceutical Research , ISSN No 2231- 6876, 2016
34. M.A .Fouad ; E.F. Elkady ; forced degredation study and validated stability indicating RP-HPLC method for determination of Nilotinib in bulk and capsule, Acta Chromatographica, 26(2014)4,637-647, DOI 10.1556/A chrome
35. G Chaitanya ; K.M.Pawar ; Development and validation of UV spectrometric method for the determination of Nilotinib Hydrochloride, Journal of Chemical and Pharmaceutical Research, ISSN No 0975-7384,2015
36. Srikanth Munipalli ; Mariappan Soundarpandian ; Tulsirao V ; V S Prakash; Reddy T ; Jeenet Jayachandran ; Manish Gangrade ; QbD approach ,RP-HPLC method for estimation of Nilotinib Hydrochloride and impurities in drug substances, American journal of Pharmtech Research, ISSN No 2249-3387, (vol 6),feb 2016
37. Elisa pirro; Silvia Die Francia; Carmen Fava; Francesco Di Carlo;a new HPLC- UV validated method for therapeutic drug monitoring of Tyrosine Kinase Inhibitors in Leukemic patients,Journal of Chromatographic Science, (vol 49) Nov-Dec 2011
38. Sudhakar Babu Kundra;Venkatramanna medreddy; Narayana reddy papadasu; Latha Jonnalagad; a validated stability indicative UPLC method for the Nilotinib hydrochloride for determination of process related and degredation impurities
,Journal Of Chromatographic Science,july 2013
39. Stephane Bouchet ; Simultaneous determination of nine tyrosine kinase inhibitors by 96-well solid-phase extraction and ultra performance LC/MS-MS,Clinica Chimica Acta,vol 412,12 may 2011,pp-1060-1067
40. Silvia Di Francia ; New HPLC–MS method for the simultaneous quantificationof the antileukemia drugs imatinib, dasatinib, and nilotinib in human plasma,Journal of chromatography, vol 877,june 2009,pp 1721-1726
41. Nilotinib drugbank DB04868

42. Nilotinib/Wikipedia /the free encyclopedia
43. Nilotinib//Medline plus.drug information
44. International Conference On Harmonization (ICH), (1997), Q2b: Validation OfAnalytical Procedure: Methodology, USFDA Federal Register, (Vol. 62), 27463
45. International Conference On Harmonization (ICH), (1995), Q2a: Text OnValidation Of Analytical Procedure,USFDA Federal Register, (Vol 60), 11260
46. B.K. Sharma ; Instrumental Methods of Chemical Analysis, 25th Edn. Meerut,(1983) S82
47. D. A.Skoog ; F.J. Holler ; S.R. Crouch ; Principle of Instrumental Analysis, 6thEdn. (2007). 893-900
48. A.H.Beckett ; J.B.tenlake ; Practical Pharmaceutical chemistry, Part II, 4th Edn.(2005) 384