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A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF NILOTINIB IN BULK AND PHARMACEUTICAL FORMULATIONS

J. Maheshwari[1]*, Allakonda Rajamani[2] Dr. A. Yasodha[3] and Jabeen[1]

[1]Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001.

[2]Associate Professor, Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001.

[3]Principal, Professor, Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001.

***Corresponding Author: J. Maheshwari**
Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Thirumala Hills, Centre City, Appannapally, Mahabubnagar, Telangana 509001.

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Nilotinib in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (35:65) v/v as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Nilotinib was found to be 2.276min. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Nilotinib in bulk and marketed pharmaceutical dosage form dosage form.

KEYWORDS: Nilotinib, RP-HPLC, Validation, Precision, ICH Guidelines.

INTRODUCTION

Nilotinib, also known as AMN107, is a tyrosine kinase inhibitor under investigation as a possible treatment for chronic myelogenous leukemia (CML). A Phase I clinical trial in 2006 showed that this drug was relatively safe and offered significant therapeutic benefits in cases of CML which were found to be resistant to treatment with imatinib (Gleevec), another tyrosine kinase inhibitor used as a first-line treatment for CML. Nilotinib[1] is a kinase inhibitor used for the chronic phase treatment of Chronic Myeloid Leukemia (CML) that is Philadelphia chromosome positive and for the treatment of CML that is resistant to therapy containing imatinib. For the potential treatment of various leukemias, including chronic myeloid leukemia (CML). Nilotinib[2] is a transduction inhibitor that targets BCR-ABL, c-kit and PDGF, for the potential treatment of various leukemias, including chronic myeloid leukemia (CML). Nilotinib is used to treat certain types of chronic myeloid leukemia (CML; a type of cancer of the white blood cells) who have recently found to have this condition in adults and children 1 year of age and older. The IUPAC name of Nilotinib[3] is 4-methyl-N-[3-(4-methyl imidazol-1-yl)-5-(trifluoromethyl) phenyl]-3-[(4-pyridin-3-ylpyrimidin-2-yl) amino] benzamide. The Chemical Structure of Nilotinib is follows

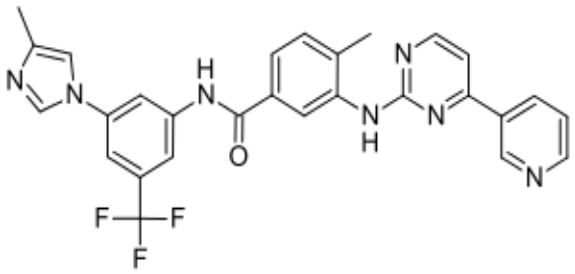

Fig-1: Chemical Structure of Nilotinib.

Literature survey[28-33] revealed LC-MS/MS and UPLC-MS/MS method to quantify Nilotinib, only few HPLC methods was reported for the estimation of Nilotinib in bulk and pharmaceutical dosage forms. Hence attempts were made to develop a simple, rapid, precise, and accurate reverse phase chromatographic method to estimate Nilotinib in bulk and pharmaceutical dosage form. The proposed method was optimized and validated as per ICH guidelines.

The main objective is to give an overview of the mechanism of Reversed -Phase High Performance Liquid Chromatography and to explain the basis of the retention mechanism and achieve high-speed separation without loss of reproducibility.

EXPERIMENTAL

Instruments Used

Table 1: Instruments used.

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals Used

Table 2: Chemicals used.

S.No.	Chemical	Brand names
1	Nilotinib	Novartis
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Method Development

Preparation of standard solution

Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Nilotinib stock solutions[4] into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.[27]

Mobile Phase Optimization

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

Optimization of Column

The method was performed with various C18 columns[11] like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Buffer and Mobile Phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.[5]

Preparation of Mobile Phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.1ml of the above Nilotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.[6]

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve

it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.1ml of the above Nilotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution
Weight 10 mg equivalent weight of Nilotinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Nilotinib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure
Inject the three replicate injections of standard and sample solutions and calculate the assay[7] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (6ppm of Nilotinib)
Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate[8] the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (8ppm of Nilotinib)
Take 0.8ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (10ppm of Nilotinib)
Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (12ppm of Nilotinib)
Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (14ppm of Nilotinib)
Take 0.14ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure
Inject each level into the chromatographic system and measure the peak area.

Plot a graph[9] of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION
Repeatability
Preparation of Nilotinib Product Solution for Precision
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Nilotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision[10] was performed on different days by maintaining same conditions.

Procedure
Analyst 1
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy
For preparation of 50% Standard stock solution
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Nilotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Nilotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Nilotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Nilotinib and calculate the individual recovery and mean recovery values.

Limit of Detection and Limit of Quantification (LOD & LOQ)
Preparation of 0.95µg/ml Solution (For LOD)
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.0095ml of the above Nilotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 2.9µg/ml Solution (For LOQ)
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.029ml of the above Nilotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Nilotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Nilotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition
The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead (35:65), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.[12-14]

RESULTS AND DISCUSSION

Method Development

Optimized Chromatogram (Standard)

Mobile phase ratio	:	Methanol: Phosphate Buffer (35:65) V/V
Column	:	Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature	:	Ambient
Wavelength	:	235nm
Flow rate	:	1ml/min
Injection volume	:	10µl
Run time	:	8min

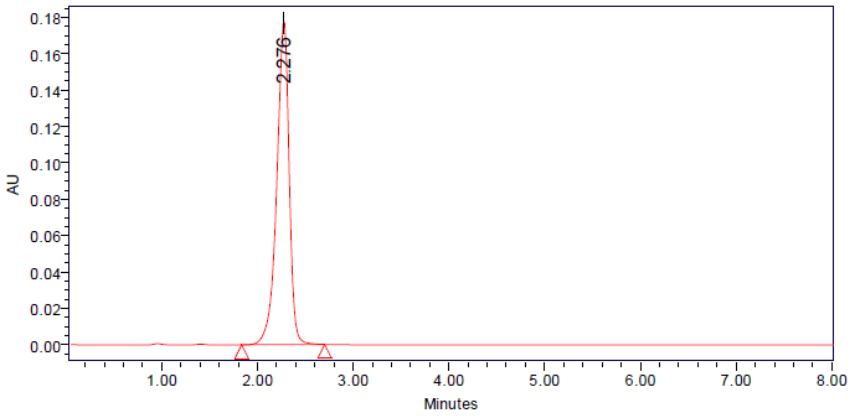

Fig. 2: Optimized Chromatogram (Standard).

System Suitability

Table 3: Results of System Suitability for Nilotinib.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nilotinib	2.277	1652847	185647	6589	1.24
2	Nilotinib	2.277	1653658	186254	6587	1.26
3	Nilotinib	2.267	1654521	185475	6584	1.28
4	Nilotinib	2.265	1653564	186594	6582	1.29
5	Nilotinib	2.277	1658745	185684	6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
% RSD			0.142371			

Specificity

The ICH documents define specificity[15] as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Nilotinib in drug product.

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.40%

The % purity of Nilotinib in pharmaceutical dosage form was found to be 99.40%.

Linearity

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Table 4: Data for Linearity of Nilotinib.

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

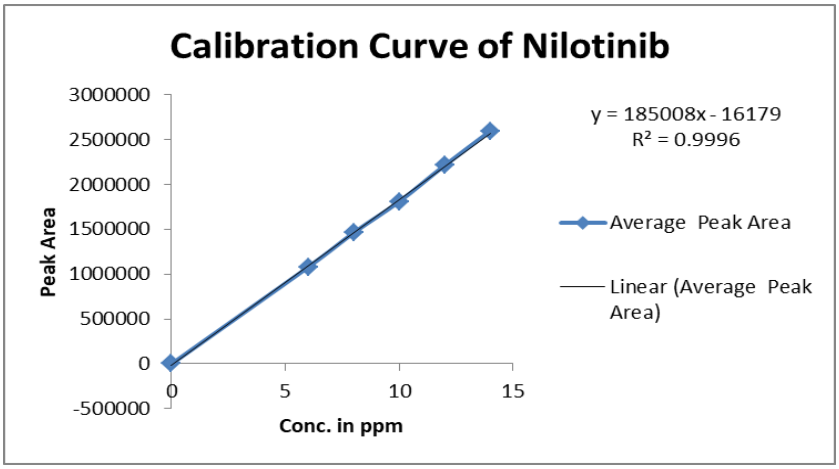

Fig. 3: Linearity Curve of Nilotinib.

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Nilotinib is a straight line.
Y = mx + c
Slope (m) = 18500
Intercept (c) = 16179
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 0.16179. These values meet the validation criteria.

Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded[16-17] the peak areas and calculated % RSD.

Table 5: Results of repeatability for Nilotinib.

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nilotinib	2.293	1658954	186958	1.26	6785
2	Nilotinib	2.276	1658745	187548	1.27	6854
3	Nilotinib	2.286	1659865	189854	1.26	6852
4	Nilotinib	2.277	1653254	186985	1.25	6784
5	Nilotinib	2.280	1654781	189542	1.24	6895
6	Nilotinib	2.293	1661324	187586	1.28	6965
Mean			1657821			
Std.dev			3120.433			
%RSD			0.188225			

Intermediate Precision
Analyst 1
Table 6: Results of Intermediate Precision for Nilotinib.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nilotinib	2.274	1678541	186589	6587	1.26
2	Nilotinib	2.258	1685985	186598	6321	1.26
3	Nilotinib	2.267	1685745	186985	6385	1.25
4	Nilotinib	2.270	1685987	187854	6580	1.26
5	Nilotinib	2.264	1698526	187549	6721	1.27
6	Nilotinib	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std. Dev.			6463.466			
% RSD			0.383182			

Analyst 2
Table 7: Results of Intermediate Precision Analyst 2 for Nilotinib.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Nilotinib	2.277	1665847	167481	6854	1.25
2	Nilotinib	2.255	1658989	167854	6785	1.26
3	Nilotinib	2.265	1659845	167895	6854	1.24
4	Nilotinib	2.255	1665964	167854	6895	1.26
5	Nilotinib	2.253	1659863	168585	6459	1.25
6	Nilotinib	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std. Dev.			3501.766			
% RSD			0.210601			

Accuracy: Accuracy[18] at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 8: The Accuracy Results for Nilotinib.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

Limit of Detection

The detection limit[19] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

= 0.95µg/ml

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve[20]

Result

= 2.9µg/ml

Table 9: Results of LOD & LOQ.

LOD	0.95925344
LOQ	2.906828605

SE of Intercept = Excel Function (Data Analysis → Regression)

SD of Intercept = SE of Intercept * √N

LOD = 3.3 * (SD of Intercept/Slope)

LOQ = 10 * (SD of Intercept/Slope)

Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Nilotinib. The method is robust only in less flow condition. The standard of Nilotinib was injected by changing the conditions of chromatography.[21] There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.[22]

Table 10: Results for Robustness.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

Stability Studies

The specificity[23-26] of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient.

Table 11: Results of Forced Degradation Studies for Nilotinib.

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1658242	0	100%	100%
2	Acidic	1331734.15	19.69	80.31	100%
3	Basic	1594233.85	3.86	96.14	100%
4	Oxidative	1394747.34	15.89	84.11	100%
5	Thermal	1575827.37	4.97	95.03	100%
6	Photolytic	1345331.73	18.87	81.13	100%
7	Water	1360090.08	17.98	82.02	100%

SUMMARY AND DISCUSSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 235nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry ODS C18 (4.6×250mm, 5µm) because it was giving good peak.

Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory

retention time. Mobile phase is Methanol: Phosphate Buffer pH-3.6 in the ratio of 35:65 v/v was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 8min because analyze gave peak around 2.276 and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of the Nilotinib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

BIBLIOGRAPHY

1. https://go.drugbank.com/drugs/DB04868
2. https://pubchem.ncbi.nlm.nih.gov/compound/Nilotinib
3. https://en.wikipedia.org/wiki/Nilotinib
4. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, 2002; 1-7.
5. A. Braith Wait and F. J. Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, 1996; 1-2.
6. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, 1997; 24-37.
7. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A John Wiley & Sons, Inc., Publication, 2007; 15-23.
8. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
9. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, 2004; 7-8.
10. Sahajwalla CG a new drug development, Vol 141, Marcel Dekker Inc., New York, 2004; 421–426.
11. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
12. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
13. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
14. Detectors (online) ,URL:http://www.dionex.com/en us/webdocs/64842-31644-02_PDA-100.pdf
15. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
16. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf
17. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
18. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, Vol 60. IFPMA, Switzerland, 1995; 1126.
19. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996; 1- 8.
20. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
21. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
22. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, 1997; 180-182.
23. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers, 1994; 1-5.
24. Sharma B K, Instrumental method of chemical analysis Meerut, 1999; 175-203.
25. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology, 2003; 5: 110-114.
26. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental Methods of Analysis" 7th edition CBS publisher and distributors, New Delhi, 1991; 436-439.
27. ICH Q2A, "Validation of Analytical Methods, Definitions and Terminology", ICH Harmonized tripartite guideline, 1999.
28. Archana Barla*, Kiran Kumar Buralla, Development and Validation of RP-HPLC Method for the Estimation of Nilotinib in Bulk and Pharmaceutical Dosage form, International Journal of Pharmaceutical Investigation, Jul-Sep, 2020; 10(3): 364-367.
29. Ramu Ivaturi1, T. Manikya Sastry2, S. Satyaveni1, Development and Validation of a Stability Indicating HPLC Method for the Determination of Nilotinib Hydrochloride in Bulk and Pharmaceutical Dosage Form, International Journal of Pharmacy and Pharmaceutical Sciences, 8(9): 41-48.
30. L. Satyanarayana, S.V. Naidu, M. Narasimha Rao, Reddy Suma Latha, The Estimation of Nilotinib in Capsule dosage form by RP-HPLC, Asian Journal of Pharmaceutical Analysis, Oct-Dec, 2011; 1(4): 100-102.
31. Dara Varun Kumar1 and Kumaraswamy Gandla2, Method Development and Validation of Anticancer Drugs (Nilotinib and Gemcitabine) by RP-HPLC Method, World Journal of Pharmaceutical and Medical Research, wjpmr, 2021; 7(11): 150–160.
32. Ramana Reddy Gopireddy, Arthanareeswari Maruthapillai, A stability indicating method development and validation for separation of process related impurities and characterization of unknown impurities of tyrosine kinase inhibitor Nilotinib by

RP-HPLC, NMR spectroscopy and ESI-MS, Materials today: Proceedings, 2021; 40(1): S127-S138.

33. Azra Takhvar 1, Somaye Akbari 1, Effat Souri 1, Reza Ahmadkhaniha 2, Ali Morsali 3, Mohammad Reza Khoshayand 4, Mohsen Amini 1, Development and validation of RP-HPLC method for simultaneous quantification of the anticancer agents, Nilotinib and Sorafenib: Application in In-vitro analysis, Progress in Chemical and Biochemical Research, 2022; 5(1): 44-52.