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RESEARCH ARTICLE

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Development and Validation of RP-HPLC for Estimation of Neratinib in Bulk and Tablet Dosage Form

M Lakshmi Kanth*, B Raj Kamal

Mewar University, NH-79, Gangrar, Chhitorgarh-312901, Rajasthan, India

ABSTRACT

An accurate RP-HPLC method developed for the estimation of Neratinib in bulk and tablet dosage form. The method is and validated for parameters linearity, accuracy, suitability, specificity, precession, LOD, LOQ and robustness. An Altima column (150 mm × 4.6 mm × 5µ) used for chromatographic separation within a runtime of 6 min. The mobile phase buffer (monopotassium phosphate) and acetonitrile (60:40 v/v) with 0.1% formic acid is used. The flow rate maintained at 1.0 ml/min with the effluents monitored at 215 nm. The Neratinib analyzed at retention time of 4.001. The concentration linear over 30-180µg/ml with regression equation y = 6065.6x + 795.43 and regression co-efficient 0.999.

Keywords: Neratinib, antineoplastic activity, ICH Guidelines, method validation.

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***Corresponding author**: Mr. M. Lakshmi Kanth
Address: Mewar University, NH-79, Gangrar, Chhitorgarh-312901, Rajasthan, India
Tel.: +91-7675851984
E-mail ✉: kanthmsc06@gmail.com

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INTRODUCTION

Neratinib 6, 7-disubstituted-4-anilinoquinoline-3-carbonitrile that exhibits antineoplastic activity and acts as inhibitor of the HER-2. [1] Neratinib reduces auto phosphorylation in cells by binding to HER-2 receptor irreversibly. [2-3] Phase I examination of single agent Neratinib administration showed a C_{max} of 5.8-119 ng/mL over a 40 to 400 mg dose range. [4-7] Chemically Neratinib is (E)-N-[4-[3-chloro-4-(pyridin-2-ylmethoxy) anilino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethyl amino)but-2-formula $C_{30}H_{29}ClN_6O_3$ and molecular weight 557.051 g/mol (Figure 1). Literature survey reveals that few UV Spectroscopy, fluorescence spectroscopy, HPLC and LC-MS/MS methods [8-13] were developed for determination of Neratinib in both human plasma and pharmaceutical dosage forms. The present research is novel and validated as per ICH guidelines. [14]

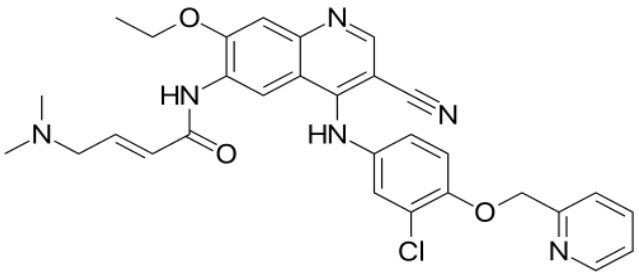

Fig. 1: Structure of Neratinib

MATERIALS AND METHODS

Neratinib was kindly gifted from Hetero Drugs Ltd. Hyderabad. Commercially available NERLYNX® (Neratinib 4 mg) tablet procured from local market. Acetonitrile (Finar reagents), monopotassium phosphate and phosphoric acid (S D fine Chem limited), formic acid (Fisher scientific) were used without further purification.

Quantitative HPLC used is of Waters Alliance 2695 system with Empower-2 Software. An Altima column (150 mm × 4.6 mm × 5µ) selected for elution of drug. The UV Spectrometer PG Instruments T60 used for analysis.

Preparation of Neratinib Standard Solution

About 0.012 g of Neratinib working standard accurately weighed into a 10 ml standard flask. Contents dissolved in 7ml of diluent and sonicated for 25-30 minutes. The contents made up to the mark with diluents. This is considered as the stock solution.

Preparation of Neratinib Working standard

10 ml of prepared stock solution taken into 100 ml volumetric flask, made up to mark with diluents to obtain Neratinib working standard of 120µg/ml.

Preparation of Neratinib Sample

Neratinib capsule powder weight equivalent to 12 mg of Neratinib taken in 10 ml volumetric flask. Diluents added till the mark and sonicated for 20-30 min and filtered. 1 ml of stock solution taken into 10 ml volumetric flask, made up to 10 ml with diluents to produce 1200µg/ml.

Preparation of Buffer

The buffer prepared by mixing 1.36 g of monopotassium phosphate with 900 ml of milli-Q water. Contents degassed and made up to 100 ml volume with water. Phosphoric acid used to adjust the pH of contents to 3.0. 100 mL of formic acid mixed with 100 mL of water to obtain 0.1% formic acid.

HPLC Conditions

The solvent A comprises of 0.01M disodium hydrogen orthophosphate (pH 3.0), Solvent B comprises of Acetonitrile with 0.1% formic acid. The contents mixed thoroughly, filtered through poly-tetra-fluoro ethanol (PTFE) filter of 0.45µm pore size. The contents degassed by sonication. The eluents monitored at 215 nm with a run time maintained at 6 min. The column must be equilibrated for 25-30 min prior to the sample analysis.

Analytical Method validation

Method validation includes testing of system suitability, specificity, linearity, accuracy, precision and robustness of the method developed.

Linearity

The ability of developed procedure obtain test results proportional to concentration of sample is measured. The method is considered valid if the correlation coefficient (r^2) > 0.998. The linearity of Neratinib was evaluated for concentrations of 30-180µg/ml.

Specificity

The analysis results of samples spiked with impurities, degradation products were compared to the results of pure samples containing no impurities or degradation products to analyse the specificity of the developed HPLC method.

System suitability

The evaluation of area under curve, resolution, retention time, tailing factor and plate number was performing to check the system suitability. The %RSD for system suitability parameter of Neratinib are within acceptable limit.

Precision

The intraday and interday precision of Neratinib standard and sample determined and results expressed in %RSD to check the precision of developed method. The interday and intraday precession studies of Neratinib carried out at different concentrations (30, 90 and150µg/ml) and results found within the acceptable limit

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOQ and LOD of the developed method were evaluated by considering parameters like signal-to-noise ratio (S/N) and peak-to-peak ratio around the RT of Neratinib. The S/N ratio of 3 is accepted for LOD and a value of 10 is accepted for LOQ. The LOD (0.43 ppm) and LOQ (1.31 ppm) values of Neratinib were within the acceptable limit.

Robustness

The capacity of developed method to stay unaffected by minimal variations in method parameters is analysed. The samples analysed by varying different optimized parameters like detection wavelength, flow rate, injection volume and mobile phase composition. The effect of all these parameters determined.

RESULTS

Linearity

Five standard solutions of Neratinib of concentrations 30-180µg/ml were injected and results tabulated (Table 1). A graph of concentration vs peak are plotted with slope of 6065.6, Y-intercept 795.4 and r^2 (correlation co-efficient) of 0.999. Regression equation of Neratinib was y = 6065.6X+795.4 (Figure 2).

Specificity

Specificity was tested by evaluating chromatogram of blank run and standard Neratinib (Figure 3, 4). These showed that no peaks, interfering peaks or baseline noise was observed.

System Suitability

The system suitability of developed method was analyzed by injecting the Neratinib working standard six times. The suitability of method evaluated from percentage relative standard deviation of RT, USP plate count, peak area and USP tailing factor (Table 2). [14]

Assay studies

Six homogeneous samples of Neratinib sample and standard analysed. The % assay of the Neratinib in the formulation estimated. The Neratinib sample and standard RT were similar i.e. 4.001. [10] From Table 3, it was found that the method is linear for concentration

30-180µg/ml for Neratinib. The % assay of marketed Neratinib drug found to be 99.76% (Table 3).

Table 1: Calibration data of Neratinib

Linearity Level (%)	Concentration (µg/ml)	Area
25	30	259635
50	60	358945
75	90	448155
100	120	685499
125	150	948512
150	180	1099245

Table 2: System suitability data of Neratinib

S. No	Name of the drug & conc (120µg/ml)	RT (min)	Peak Area	USP Plate Count	USP Tailing
1	Neratinib (std)	4.001	730064	7325	1.69
2	Neratinib (std)	4.002	723381	7386	1.68
3	Neratinib (std)	4.003	721505	7291	1.69
4	Neratinib (std)	4.004	720970	7243	1.68
5	Neratinib (std)	4.008	726077	7254	1.69
6	Neratinib (std)	4.013	719446	7602	1.67
Mean			723574		
Std. Dev.			3912.1		
%RSD			0.5		

Table 3: Assay data Neratinib

Sample No	Retention time	%Assay
1	4.001	99.89
2	4.003	99.40
3.	4.008	99.44
4.	4.013	100.04
5.	4.004	100.16
6.	4.002	99.61
Avg	4.005	99.76
SD		0.3166
%RSD		0.32

Table 4: Intra-day precision data of Neratinib

Level	Low	Middle	High
Concentration (µg/ml)	**30**	**90**	**150**
Peak area Session 1	256985	445698	948565
Session 2	263845	448756	958925
Session 3	254416	458951	945698
Avg. peak area	258415.33	451135	951062.66
SD	4874.51	6939.39	6958.242
%RSD	1.88	1.53	0.73

Table 5: Inter Day precision data of Neratinib

Level	Low	Middle	High
Concentration(µg/ml)	**30**	**90**	**150**
Peak area day 1	255894	446985	945845
day 2	248556	458911	958589
day 3	252697	455892	945289
Avg. peak area	252382.3	453929.3	949907.66
SD	3679.10	6200.51	7523.3
%RSD	1.45	1.36	0.79

Table 6: Accuracy data of Neratinib

S. No	Spiked level	% Recovery	% RSD
1	80%	99.38	
2	100%	100.02	0.52
3	120%	98.99	

Precision

Intra-day precession was performed for concentration levels 30µg/ml, 90µg/ml and 150µg/ml within the same day at three different times session 1, session 2 and session 3.

Inter-day precision was carried by conducting at different concentration 30µg/ml, 90µg/ml and 150µg/ml level on three different days, using same homogeneous samples.

The %RSD values for both inter-day and intra-day precision were found within acceptable limit. Results tabulated in Table 4 and Table 5.

Table 7: Robustness data of Neratinib

Parameter	%RSD
Flow Minus	0.6
Flow Plus	0.5
Mobile phase Minus	0.4
Mobile phase Plus	0.6
Temperature minus	0.4
Temperature plus	0.5

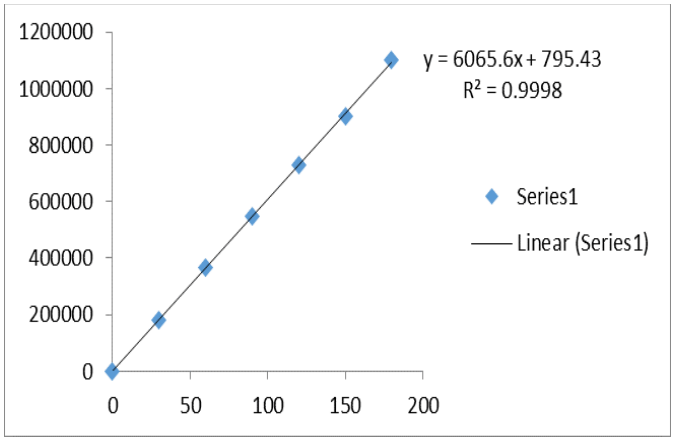

Fig. 2: Calibration Curve of Neratinib

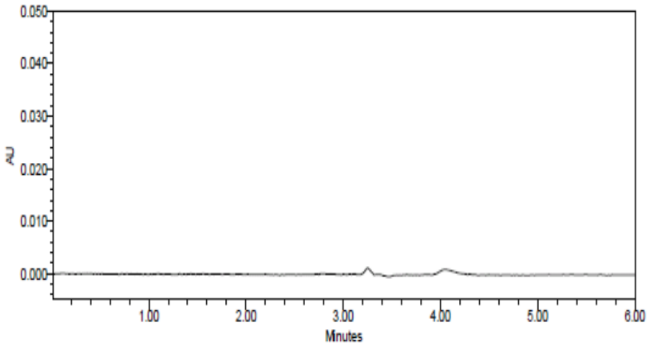

Fig. 3: Blank chromatogram

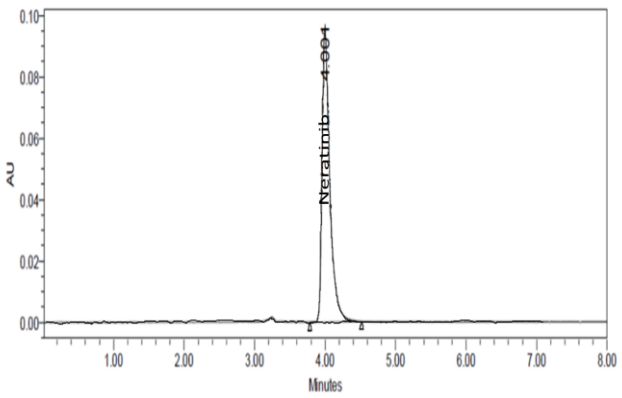

Fig. 4: Chromatogram of Neratinib standard

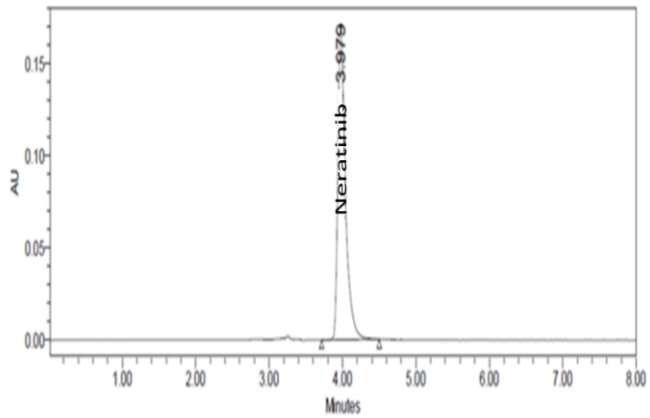

Fig. 5: Chromatogram of Neratinib sample

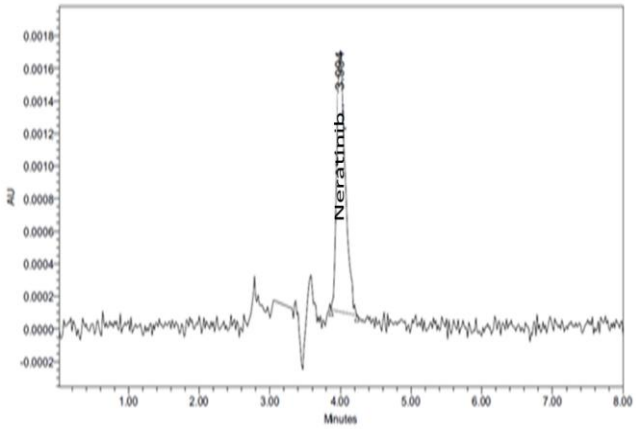

Fig. 6: LOD Chromatogram of Neratinib

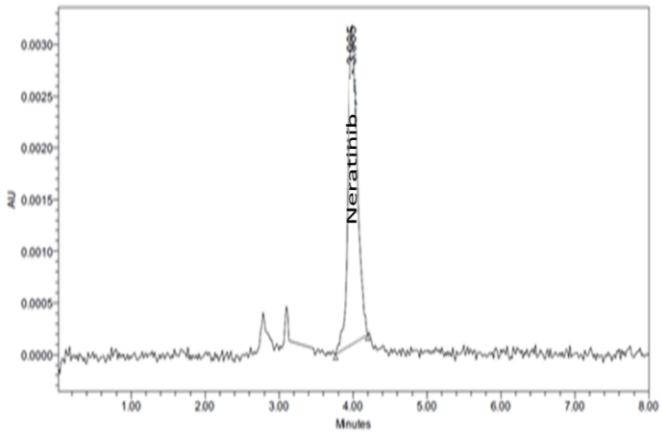

Fig. 7: LOQ Chromatogram of Neratinib

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOQ and LOD values for Neratinib were 0.43ppm and 1.31ppm respectively (Figure 6 and Figure 7).

Accuracy

The accuracy of developed method is determined for three concentration levels of 80%, 100% and 120% by recovery experiment. The results established by performing recovery studies on three replicates of three concentrations by adding known amount of Neratibin. Results found within the acceptable limit (Table 6).

Robustness

Six samples in each case analysed by altering flow rate, mobile phase composition and column temperature. The %RSD in each case evaluated and results were within acceptable limits (Table7).

DISCUSSION

A simple, accurate and linear RP-HPLC method developed for estimation of Neratinib. The mobile phase comprises of buffer (potassium dihydrogen orthophosphate) and acetonitrile in the ratio of 60:40 (v/v) with 0.1% formic acid with retention time of 4.001 min. The developed method was validated for various parameters as per ICH guidelines. The linearity of developed method was established for concentration of 30-180µg/ml with regression coefficient value of 0.9998 indicating high precision of the method. The system suitability data are within the acceptable limit. The percentage assay of Neratinib was 99.76% indicating non-interference of the common excipients used in the formulation. The limit of detection (LOD) and limit of quantification (LOQ) for Neratinib were 0.43ppm and 1.31ppm respectively.

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