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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF NEVIRAPINE IN BULK AND DOSAGE FORMS

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ABSTRACT

An economic, accurate, precise and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for Nevirapine. It is an anti-viral drug and it is belongs to the class of non nucleoside reverse transcriptase inhibitor (NNRITS) available in market in the form of tablets and oral suspension. The quantification of Nevirapine was carried out by using Agilent 150 column as stationary phase with the mobile phase consisting of methanol and HPLC grade water in the ratio of 50:50(v/v) which resulted in best sensitivity. The mobile phase was pumped at a rate of 1ml/min. The drug was identified with PDA detector at 229.0 nm. The processed method was validated in terms of linearity, range, precession, robustness, accuracy as per ICH guidelines. The validation of processed method was verified by recovery studies and can be applicable in routine determination of Nevirapine in pharmaceutical formulations.

Keywords: Nevirapine, non nucleoside reverse transcriptase inhibitor and RP-HPLC.

INTRODUCTION[1-17]

Nevirapineis a anti-viral medicine belonging to the class of non nucleoside reverse transcriptase inhibitor (NNRITS) with activity against the human immunodeficiency virus type-1 (HIV-1). It prevents the multiplication of HIV in human body. Nevirapine binds directly with reverse transcriptase and blocks the RNA dependent and DNA dependent, DNA polymerase activities by causing a disruption of enzyme's catalytic site. Thus inhibits the cycle DNA synthesis and prevents the viral replication. Mono therapy of these NNRITS leads to resistance due to single mutation in viral reverse transcriptase enzyme. Hence, they should be taken in combination with other HIV medication such as Retonavir or videx.

Nevirapine is well absorbed orally and it has the bioavailability about 90-95%. It is a lipophilic drug and widely distributed throughout the body. It is metabolized in liver by CYP3A4 enzyme and excreted in urine. The half life of drug is 25-30 hours.

Chemically Nevirapine is 2-cyclopropyl-7-methyl-2,4,9.15tetraaztricyclo [9.4.0.0^{3,}"] pentadeca-1(11),3,5,7,1,2,14-hexane-10-one.chemical formula is $C_{15}H_{14}N_4O$ with the molecular weight of 266.2979. The trade name of Nevirapine is Viramune.

Structure

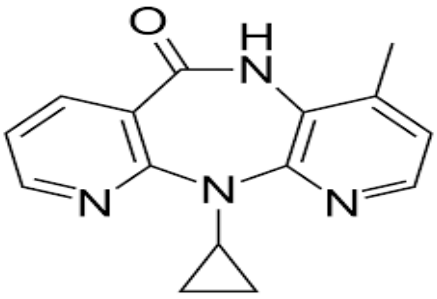

Fig. 1: Structure of Nevirapine

In this research work we have developed, optimized, and validated the method using RP-HPLC. Drug was assayed by validated method. The main objective of this method is to develop time saving and cost effective.

MATERIALS AND METHOD

CHEMICALS AND REAGENTS

Reference standard was obtained from sigma Aldrich laboratories. The formulation used for assay is Nevirapine manufactured by Viramune and solvents used in this method were acetonitrile and HPLC grade water.

INSTRUMENTATION

Method development and validation was carried out by RP-HPLC (Shimadzu) with PDA detector module with auto-sampler. Column used was Agilent Eclipse XBD (150*4.6 * 5µm), and data recorded using LC Solutions software.

DILUENT

Methanol: Water (50:50).

PREPARATION OF STANDARD

Weigh accurately about 100mg of drug and transfer it into 10ml volumetric flask and make up the volume up to 10ml using diluent and sonicated for 5 minutes. Pipette out 1ml of above solution into 10ml volumetric flask, make up the volume with diluent. Obtained standard concentration is 100µg/ml.

PREPARATION OF SAMPLE

Take 20 tablets and triturate it. Weigh accurately equivalent to 100mg of tablet powder and transfer it into 10ml volumetric flask and make up the volume up to 10ml using diluent and sonicated for 5 minutes. Pipette out 1ml of above solution into 10ml volumetric flask, make up the volume with diluent.

METHOD OPTIMIZATION

Based on the literature of Nevirapine and its combinations, one method was developed after conducting several trials and developed method was optimized.

VALIDATION

The developed method was validated foe different parameters like system suitability, Linearity, accuracy, precision, LOD, LOQ, and Robustness as per ICH guidelines.

SYSTEM SUITABILITY

By injecting it six times into the system, the chromatograms of 100µg/ml were analyzed. Form chromatogram the system suitability parameters like plate count, tailing factor, capacity factor and reproducibility were determined.

LINEARITY

A series of solutions were prepared at concentration levels as 50 µg/ml, 75 µg/ml, 100µg/ml, 125 µg/ml,and 150µg/ml. A 10µlvolume from each concentration of solutions were injected thrice into the HPLC system. Chromatograms were recorded under optimized chromatographic conditions. A graph was plotted considering peak areas on Y-axis and concentration on X-axis. The linear equation, Y-intercept, slope of regression line and regression constant (r^2) were calculated.

PRECISION

Repeatability or intra-day precision: The peak areas of 100 µg/ml were analyzed on the same day by injecting it six times into the system. The chromatogram was recorded and RSD was calculated.

ACCURACY

A series of solutions were prepared in triplicate by spiking the known standard concentrations of Nevirapinein the range of 50-150% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 50, 100, and 150µg/ml of Nevirapine standard. The percentage recoveries of three different concentrations were found to be within the range of 98 to 102 % as per ICH Q_2R_1 guidelines.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

LOD and LOQ can be calculated based on the signal to noise ratio approach, visual evaluation and standard deviation of the response and slope of the calibration curve. The slope (S) is calculated from the equation of straight line in calibration curve of the analyte. The standard deviation (σ) is calculated based on its blank response or they-intercepts of regression line. Formulas were given below

LOD= (3.3X SD)/Slope
LOQ= (10 X SD)/Slope

ROBUSTNESS& RUGGEDNESS

The robustness of a method is its ability to remain unaffected under changes in parameters. Robustness was carried out by altering the flow rate (±0.2ml/min) and mobile phase (60:50 & 50:60) .The standard solution comprising of Nevirapine (100µg/ml) was injected six times and the %RSD was calculated for the resultant area of the peak.

ASSAY

Twenty tablets of Nevirapine were taken and powdered, Weigh accurately equivalent about 10 mg of lable drug and transferred into 100 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally made up the volume with diluent. The solution was then

injected into the HPLC system. The sample was prepared in triplicates.

% Assay =

$$\frac{\text{(Area of unknown X Conc of standard)}}{\text{(Area of standard X Conc of unknown)}} \text{ X 100}$$

RESULTS AND DISCUSSION

OPTIMIZATION OF CHROMATOGRAPHIC

Developed method was optimized for different parameters.

Parameters were given in Table.1 and Fig No 2.

SYSTEM SUITABILITY

The percentage area of Relative Standard deviation (RSD) from six replicate injections was found below2.0% (diluted standard solution, 100 µg/ml of NEVIRAPINE). Low values of RSD of replicated injections indicate that the system is precise. The results are presented in Table2.

LINEARITY

The calibration curve was made by plotting the concentration on X-axis against peak area on Y-axis. A series of Nevirapine standard solution were prepared in the range of 50 µg/ml-150 µg/ml. The correlation coefficient of the curve was found to be0.998with a regression equation of Y=46503x + 41001. This is shown in figure no 3 and results were given in table no 3.

PRECISION

Repeatability or intra-day precision: The peak areas of 100ug/ml were analyzed on the same day by injecting it six times into the system. %RSD was calculated. The %RSD was found to be 0.025. Results were given in table no:4.

ACCURACY

Recovery of Nevirapine was found to be 98.0% to 102.0%. The summary of % recovery of Nevirapine was mentioned in Table 5.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

LOD and LOQ were calculated from linearity graph. The limit of Detection and limit of Quantification were found out to be 0.032 ng/ml and 0.098ng/ml respectively.

ROBUSTNESS AND RUGGEDNESS

Robustness studies were performed by changing the flow rate (± 2 ml/min), column temperature (±5^{o}C) and mobile phase ratio. No significant effect was observed on system suitability parameters deliberate change such as resolution, RSD, tailing factor, or the theoretical plates of Nevirapine. Thus, the method was found to be robust with respect to variability in applied conditions.

ASSAY

Tablet solution was injected into the HPLC system for three times and % assay of drug was found to be99.2%. These results were tabulated in Table No 6.

CONCLUSION

An easy, rapid and efficient Reverse-Phase HPLC method was developed for quantitative estimation of Nevirapine in drug product and drug substance. The method was validated as per ICH Q2 (R1) guidelines. A precise, accurate, linear, robust and rugged method was found during validation. Limits of detection 0.032 ng/ml and limits of quantification 0.098ng/mlalso determined. By performing the assay of Viramune tablet the percentage purity was found to be 99.2%. Hence it was concluded that this method is useful for the determination of both pharmaceutical substance and pharmaceutical product.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors have no conflict of interests to disclose other thanwhat has been acknowledged above.

Table 1: Optimized conditions

S.NO	Parameter	Results
1	Mobile phase	Methanol and water(50:50)
2	Stationary phase	Agilant 150 column
3	λ max	229nm
4	Run time	4.034min
5	Injection volume	20µl
6	Flow rate	1.0 ml/min
7	Area	1823653
8	Height	102883
9	Tailing factor	0.995

Table 2: System Suitability Results

S.no	Area	Tailing Factor
1	1824593	0.995
2	1823653	0.984
3	1824534	0.989
4	1823892	0.993
5	1823633	0.995
6	1823625	0.996
Avg	1823988.333	
SD	456.809661	
%RSD	0.02504455	

Table 3: Linearity Results

CONC	AREA	AVERAGE
50 µg/ml	898248	898881.3
	899148	
	899248	
75 µg/ml	1289784	1289847.3
	1289934	
	1289824	
100 µg/ml	1824593	1824260
	1823653	
	1824534	
125 µg/ml	2287213	2287146.3
	2286913	
	2287313	
150 µg/ml	2725374	2725380.6
	2725294	
	2725474	

Table 4: Precision Results

S.No	RT (min)	AREA
1	4.034	1824593
2	4.034	1823653
3	4.034	1824534
4	4.034	1823892
5	4.034	1823633
6	4.034	1823625
Avg		1823988.3
SD		456.80
%RSD		0.025

Table 5: Accuracy Results

Conc Level	Area	STD Area	Conc added	Conc Recovery	% Recovery	Avg
50%	898248	1823988	50	49.25	98.49	98.56
	899148	1823988	50	49.30	98.59	
	899248	1823988	50	49.30	98.60	
100%	1824593	1823988	100	100.03	100.03	100.01
	1823653	1823988	100	99.98	99.98	
	1824534	1823988	100	100.03	100.03	
150%	2725374	1823988	150	149.42	99.61	99.61
	2725294	1823988	150	149.41	99.61	
	2725474	1823988	150	149.42	99.62	

Table 6: Assay Results

S.No	Sample Area	Standard Area
1	1809893	1824593
2	1809653	1823653
3	1809534	1824534
4	1809892	1823892
5	1809633	1823633
6	1809625	1823625
Avg	1809705	1823988.3
% Assay	**99.2**	

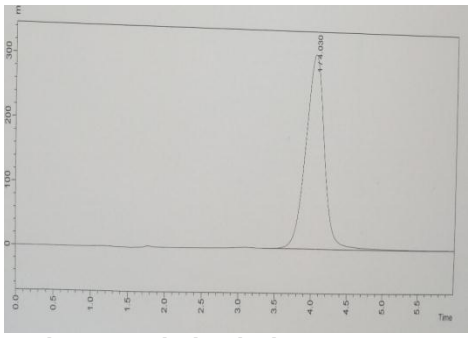

Fig. 2: Optimized Chromatogram of Nevirapine

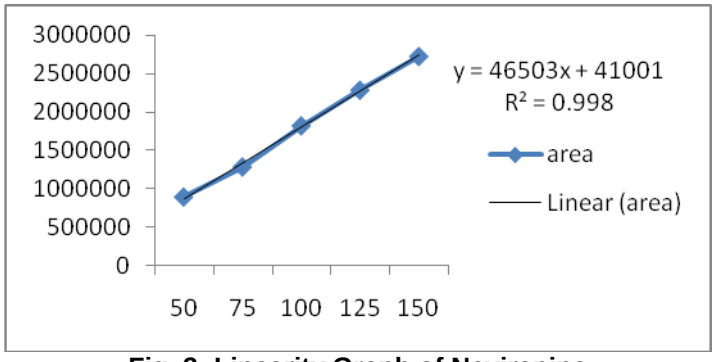

Fig. 3: Linearity Graph of Nevirapine

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