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DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL METHOD FOR THE ESTIMATION OF NEVIRAPINE IN BULK AND IN TABLET DOSAGE FORM BY RP – HPLC METHOD

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ABSTRACT

A simple, sensitive, specific RP-HPLC method was developed for the estimation of Nevirapine in bulk and pharmaceutical formulation. This method was based on HPLC separation of the drug in reverse phase mode using C_{18} column (150 mm × 4.6 mm i.d. 5µ). The mobile phase constituted of Acetonitrile: 0.01M Phosphate Buffer (PH 5.3) adjusted with orthophosphoric acid (40:60 v/v) and flow rate 1.0ml/min. Detection was performed at 220 nm. Separation completed with in 5minutes. Calibration curve was linear with the correlation coefficient was 0.9996 over a concentration range of 50 to 150µg/ml for the drug. The relative standard deviation (R.S.D) was found <2.0% for RP-HPLC method.This method have been successively applied to bulk and pharmaceutical formulation. The present method was validated according to ICH guidelines.

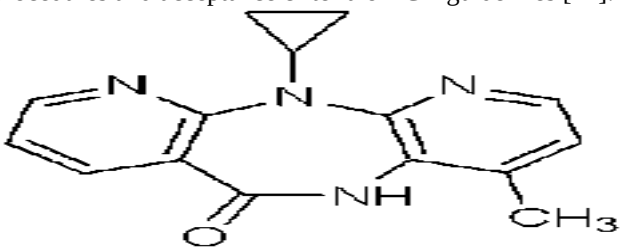
Keywords: Nevirapine, UV-spectroscopy, High Performance Liquid Chromatography.

INTRODUCTION

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds. The chemical name of nevirapine (Figure. 1) is 11-Cyclopropyl-4-methyl-5, 11-dihydro-6Hdipyrido [3, 2-b: 2′, 3′-e] [1, 4] diazepin-6-one; used to treat HIV-1infection and AIDS. A single dose of Nevirapine given to both mother and child reduced the rate of HIV transmission by almost 50%. Mechanism of action is that, NNRTIs exhibit a classical noncompetitive inhibition pattern with the enzyme. Nevirapine is readily absorbed after oral administration with a peak plasma concentration at 4 hr. Cmax for Nevirapine is 1-2 µg/ml. The concentration of the drug in the CNS is 45% of that in plasma. It crosses the placenta and has been detected in breast milk. Nevirapine undergoes extensive metabolism in the liver mainly by the cytochrome P450 isoenzymes of the CYP3A family. It is excreted via urine as the glucuronide conjugates of the hydroxylated metabolites. The drug is widely distributed in body tissues and the CNS [1-5].

A literature survey revealed that the few analytical methods available for estimation of nevirapine from pharmaceutical formulations [6-10] and from human plasma [11-16].

The reported method for the estimation of nevirapine from pharmaceutical formulations includes HPLC, Spectrophotometry and HPTLC Methods of analysis. Also the above methods are not validated for its performance under stress conditions thus rendering them unsuitable for stability studies. Thus an attempt was made to develop a new, simple, accurate and validated method for determination of Nevirapine by reverse phase high-performance liquid chromatographic method along with its stability studies. The method was validated as per the procedures and acceptance criteria of ICH guidelines [17].



The Literature survey indicates that there are very few methods for the determination of Nevirapine. Therefore an attempt was made to develop and validate a simple and economical RP-HPLC method as per ICH guidelines for the estimation of Nevirapine pharmaceutical dosage forms.

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MATERIALS AND METHOD

Nevirapine (NAV) generous gift samples from Cipla Ltd. (Mumbai, India). A commercial NEVIMUNE (Cipla) and NEVIPAN (Crosland's) tablets containing 200 mg of NEVIMUNE were purchased from a local market and used within their shelf-life period. The HPLC grade acetonitrile, methanol and water were purchased from Rankem (New Delhi, India). All other chemicals used were of pharmaceutical or analytical grade from Rankem (New Delhi, India).

Shimadzu - 1700 Double Beam UV - Visible spectrophotometer with pair of 10mm matched quartz cells, Shimadzu HPLC system(LC – 10 ATvp solvent deliver module, SPD – 10 Avp UV - Visible detector,) using Phenomenax Luna C_{18} column (150 mm × 4.6 mm i.d. 5µ), apparatus was used for the analysis. The mobile phase constituted of Acetonitrile : 0.01MPhosphateBuffer pH adjusted to 5.3 with orthophosphoric acid (40:60 v/v) and flow rate was 1.0ml/min. Detection was performed at 248nm.

RP-HPLC method

Optimized Chromatographic Conditions

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (150 mm × 4.6 mm i.d. 5µ)
Mobile phase	-	Acetonitrile: 0.01MPhosphateBuffer pH adjusted to 5.3 with Orthophosphoric acid
Proportion of mobile phase	-	40: 60 % v/v
Detection wavelength	-	220 nm
Flow rate	-	1 ml/ min
Temperature	-	Ambient
Sample load	-	20 µl
Diluent	-	Water: Acetonitrile (20:80)
Operating pressure	-	150 kgf
Method	-	External Standard
Calibration method		

The solution of Nevirapine was injected and the respective chromatogram was recorded. The chromatogram are shown in Figure 2.

Preparation of stock and standard solutions with calibration curve

Standard stock solution of Nevirapine was prepared by dissolving 50 mg of the drug in 50ml of Diluent (Water: Acetonitrile (20:80) HPLC grade). Aliquots of working standard solution (0.5 – 1.5ml) were taken and diluted with mobile phase to obtain series of solution in the concentration range of 50 - 150 µg/ml. All the solutions were injected and the chromatograms were recorded at 220 nm and calibration curve was plotted using peak area Vs concentration. The values of slope and correlation coefficient were found to be 17136 and 0.9996 respectively.

Assay of tablet formulation

Ten tablets of Nevirapine were accurately weighed and average weight of tablet formulation was determined. The tablets were crushed, the tablet powder equivalent to 50 mg of Nevirapine was transferred to a 50ml volumetric flask. Dissolve the active ingredients and volume was made up to 50ml with Diluent, the contents were sonicated for 15 minutes, centrifuged at 2000 rpm for 15 minutes and filtered through a 0.2µ membrane filter. From the clear solution, This solution was used for further analysis. 1 ml of test solution was transferred into six 10 ml volumetric flasks and made up to the mark with mobile phase. A 20µl volume of each sample solution was injected into the sample injector of HPLC six times under the chromatographic conditions as described. The peak area was measured at 220nm. The amount of drug present in the sample solutions were determined using calibration curve of standard Nevirapine. The results are shown in table 4.

METHOD VALIDATION

Linearity

The plot of absorbance against concentration is shown in Figure 3. It can be seen that plot is linear over the concentration range of 50 to 150 µg/ml for HPLC. Nevirapine with a correlation coefficient (r^2) 0.9996.

Precision

Intra day and inter day precision was determined by repeating assay three times on the same day for intra day and on different days for inter day precision. The relative standard deviation for six replicates of sample solution was less than 2.0%, which met the acceptance criteria established for spectroscopic method. The obtained results were presented in table 3.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 80,100,120% of the test concentration as per ICH guidelines and low relative standard deviation value show the accuracy of the Spectroscopy and HPLC methods. The data were presented in table 4.

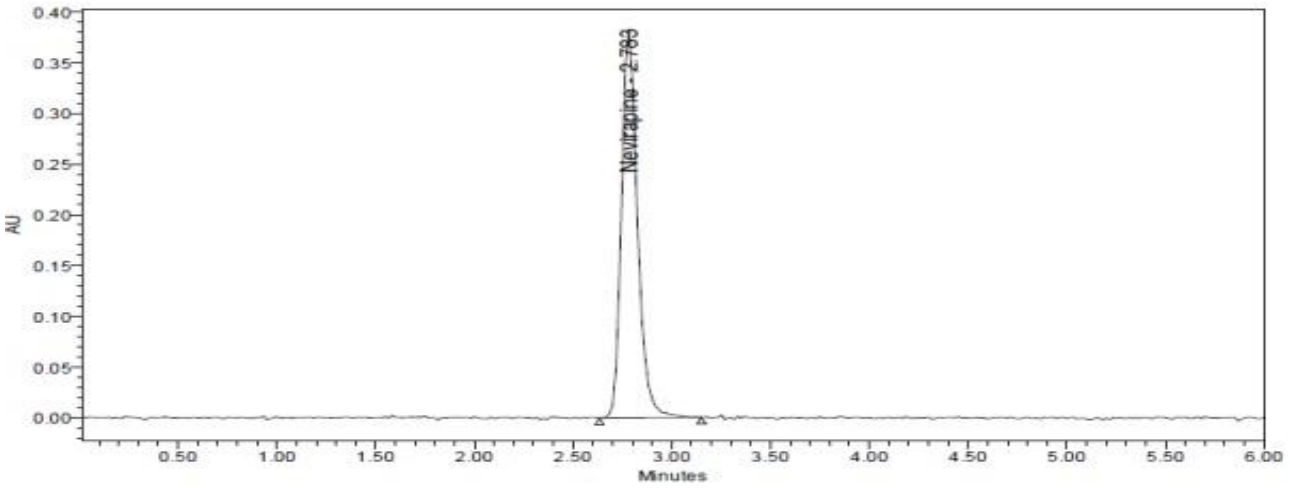
LOD and LOQ (sensitivity)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The relative standard deviation of the regression lines and slope of the calibration curve were used to calculate LOD and LOQ.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 48 hours. The relative standard deviation was found below 2.0%. It shows that standard and sample solution were stable up to 48 hours at room temperature.

Figure 1. Chromatogram of Nevirapine



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Nevirapine	2.783	2161315	100.00	5603	1.23

Figure 2. Calibration curve of Nevirapine by RP – HPLC method

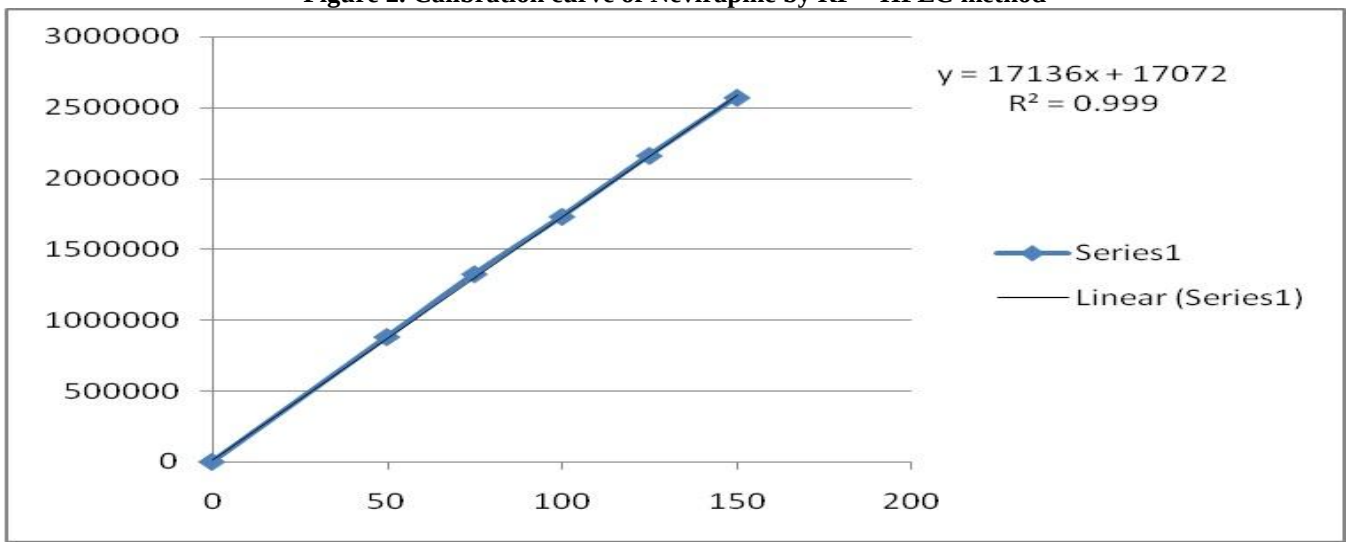


Table 1. Optical characteristics of Nevirapine by RP-HPLC method

S.No	Parameters	Observed Valves*
1	λmax (nm)	220
2	Beer's law limit (µg/ ml)	50 - 150
3	Correlation coefficient (r)	0.9996
4	Regression equation (y = mx + c)	Y = 17136x + 17072
5	Slope (m)	17136
6	Intercept (c)	17072
7	LOD (µg/ ml)	0.01576613
8	LOQ (µg/ ml)	0.047776151
9	Theoretical plates	5526
10	Tailing factor	1.21
11	Standard error	16866.339

*Mean of three observations

Table 2. Quantification of formulation - NEVIMUNE by RP – HPLC method

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
NEVI	1 2 3 4 5 6	200 200 200 200 200 200	201.3 200.5 199.8 199.6 200.9	100.65 100.25 99.91 99.81 100.06	100.275	0.3588	0.3578	0.1464

* Mean of six observations

Table 3. Inter day and Intra day analysis of formulation – NEVIMUNE by RP – HPLC method

Drug	condition	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
NEVI	Intra day	1 2 3	200 200 200	199.95 200.08 199.42	99.97 100.04 99.71	99.90	0.1563	0.1565	0.6383
NEVI	Inter day	1 2 3	200 200 200	198.05 200.25 200.51	98.87 98.45 99.44	99.8	0.6044	0.6056	0.2467

* Mean of six observations

Table 4. Recovery analysis of formulation – NEVIMUNE by RP-HPLC method

Drug	Sample No.	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated* (µg/ml)	Amount recovered (µg/ml)	% Recovery	± S.D	% R.S.D	S.E.
NEVI	1 2 3	50.05 50.05 50.05	10 20 30	60.078 70.136 80.06	10.028 20.086 30.01	101.4 102.15 100.16	±1.0050	0.9927	0.5802

*Mean of three observations

RESULTS AND DISCUSSION

In this study a simple, fast and reliable HPLC methods were developed and validated for the determination of Nevirapine in bulk and pharmaceutical formulation. As these proposed method have the lowest LOD values and wider linearity range is more sensitive method. From the results obtained, we conclude that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover, these method was simple and in expensive and this can be employed for the routine quality control of Nevirapine in bulk and pharmaceutical formulation.

CONCLUSION

Proposed study describe a new RP-HPLC method for the estimation of Nevirapine using simple mobile phase with low buffer concentration compared to the proposed methods. The method gives the short analysis time (< 5 min). The method was validated and found to be simple, sensitive, accurate and precise. The percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for the routine analysis of Nevirapine in dosage form.

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