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Development and validation of RP-HPLC method for the estimation of nevirapine in bulk drug and tablets

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ABSTRACT
A reverse phase high performance liquid chromatography [RP-HPLC] method has been developed for the estimation of Nevirapine in bulk drug and pharmaceutical dosage forms. The quantification was carried out on Octa Decyl Silane column in isocratic mode, with mobile phase consisting of methanol and acetate buffer in the ratio of 60:40[v/v].The mobile phase was pumped at a rate of 1.0 ml/min and the detection was carried out at 280 nm and the linearity was found to be in the range of 25 to 100 µg/ml. The regression equation was found to be Y=18984x +6262.9 with correlation coefficient [r^2] of 0.9998. The % recovery values were found to be in the range of 100.18-101.08%. The proposed method was validated for accuracy and precision. Statistical analysis proves that the method was found to be simple, precise, accurate, rapid and reproducible and can be used for the routine determination of Nevirapine in bulk drug and in pharmaceutical formulations.

Key words: HPLC, Nevirapine tablets.

INTRODUCTION

Nevirapine is chemically, 1-cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido [3,2-b: 2^1,3^1-e] [1,4] diazepin-6-one. It is a non-nucleoside reverse transcriptase inhibitor and antiretroviral used in the treatment of AIDS [1].Nevirapine is official in Indian Pharmacopoeia. The chemical structure of Nevirapine is shown in Fig.1. Literature survey reveals that few HPLC [2-10] methods were reported earlier for the estimation of Nevirapine in human plasma in its formulation. The main objective of the present work was to develop simple, fast, inexpensive, sensitive and accurate method which could be applied to analyse Nevirapine in pure form and in pharmaceutical dosage form.

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EXPERIMENTAL

Instrumentation

An isocratic high performance liquid chromatograph (SHIMADZU, HPLC) with LC-10ATVP, HPLC-pump K-501 and with software N4000 version 1.7 and UV/Vis detector SPD-10A (SHIMADZU) was used. Column used was C_{18} (250 x 4.6 mm; particle size 5 µm and packing material was Eurosphere-100).

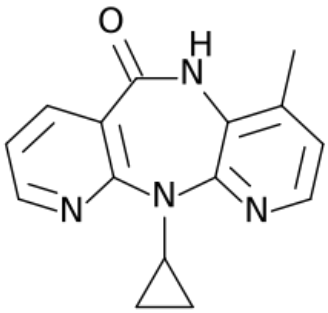

Fig. 1: Structure of Nevirapine

Chemicals and reagents

Pure drug sample of Nevirapine was received as a gift sample from Hetero

drugs Limited, Hyderabad and was used as such. The water, methanol and ammonium acetate used were of HPLC grade from Qualigens and glacial acetic acid was of analytical grade from Fischer Scientific.

Chromatographic conditions

Chromatographic separation was achieved at ambient temperature (28^0C) on a RP- HPLC by using a mobile phase consisting of methanol and acetate buffer in the ratio of 60:40 (v/v) and glacial acetic acid was added to adjust the pH 3.0. The mobile phase was pumped at a rate of 1.0 ml/min. The detector wavelength was set at 280 nm.

Working standard of drug solution

About 100 mg of Nevirapine was weighed accurately and dissolved in 100 ml of methanol in a 100 ml volumetric flask and diluted up to the mark with methanol to get the concentration of 1 mg/ml. The final concentration of the drug solution is brought to 100 mg/ml by diluting with mobile phase.

Estimation of Nevirapine from commercially available tablets by the proposed method

Commercially available tablets of Nevirapine were taken from the Indian market for the estimation of total drug content per tablet by proposed method. Twenty tablets of Nevirapine each containing 200 mg were accurately weighed; average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 50 mg of Nevirapine was transferred into 50 ml volumetric flask and dissolved in 25 ml of methanol and sonicated for 5 minutes. The solution was filtered through whatmann filter paper no.41. The residue was washed with 5 ml portions of methanol three times and the total volume of the filtrate was made up to 50 ml with methanol (1 mg/ml). The final concentration was brought 100 µg/ml with mobile phase. The solution was then analyzed after dilution by RP-HPLC method. This solution was further diluted stepwise with mobile phase in such a way that, various aliquots contain 25 to 100 µg/ml and was filtered through a 0.45 µm membrane filter. All determinations were conducted five times.

ASSAY PROCEDURE

Composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase methanol: acetate buffer 60:40 was passed through 0.45 µm membrane filter. It was delivered at 1.0 ml/min for column stabilization. During this period, the base line was continuously monitored. The wave length selected for the detection was 280 nm .The prepared dilutions containing concentrations of Nevirapine in the range of 25-100 µg/ml were injected into the column. The peak areas were recorded for all the chromatograms. The chromatogram of Nevirapine is shown in Fig.2. Calibration curve was constructed by plotting peak areas [y-axis] vs concentration [x-axis] and the linear relationship was evaluated by calculation of regression line by the method of least squares. The calibration curve of Nevirapine is shown in Fig.3.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The run time was set at 10 min and the retention time for

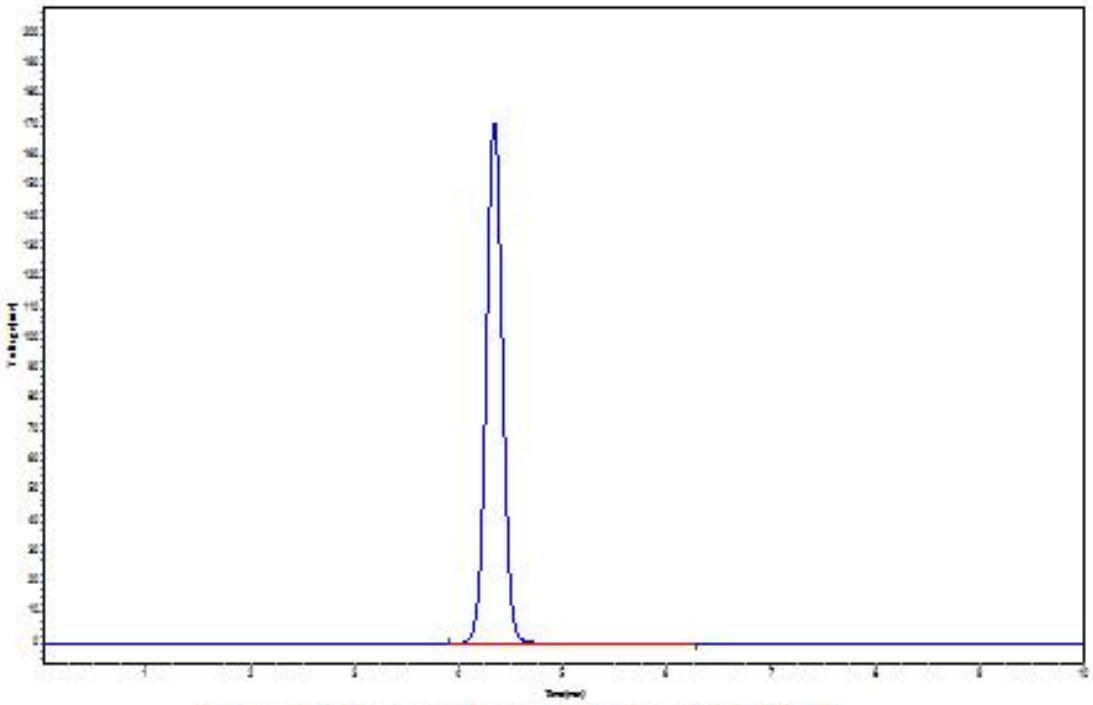

Fig. 2: Chromatogram of Nevirapine in RP-HPLC

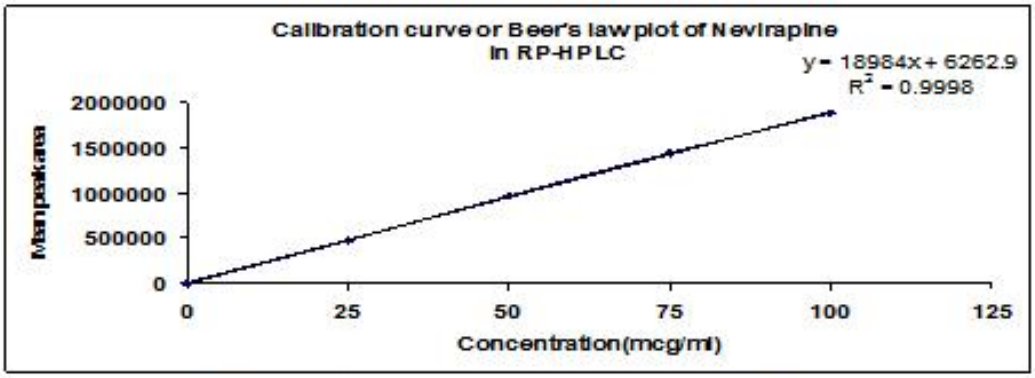

Fig. 3: Calibration curve of Nevirapine in RP-HPLC

Nevirapine was 4.33 min. Each sample was injected 5 times and the retention times were same. The peak area of the drug was reproducible as indicated by low coefficient of variance [below 2.0%]. When the concentrations of Nevirapine and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship [r^2=0.9998] was observed between the concentration of Nevirapine and the respective peak areas in the range 25-100 µg/ml. The regression of Nevirapine concentration over its peak area was found to be Y=18984x +6262.9, where Y is the mean peak area and X is the concentration of Nevirapine. The regression equation was used to estimate the amount of Nevirapine, either in tablet formulations or in validation study [precision and accuracy].

The HPLC method developed in the present study has been used to quantify Nevirapine tablet dosage forms. Nevirapine tablets [each containing 200 mg of the drug] were analyzed as per the procedure described above .The average drug content was found to be 99.68% of the labeled amount The results of analysis are expressed in

Table 1. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed reverse phase HPLC method.

HPLC method was also validated for intra and inter-day precision. When the solution containing 50 µg/ml of

Nevirapine was repeatedly injected on the same day, the %RSD in the peak area for five replicate injections was

Table 1 – Results of Analysis

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	200	199.36	99.68	0.65

*Average of five determinations.

Table 2 Results of Recovery studies

Brand used	Label claimed (mg)	Mean assay value	Known amount of Nevirapine added	Mean % recovery ±%RSD*
Tab-a	200	99.68	10mg	100.18±0.67

Table 3 Results of Intraday precision studies

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	200	199.32	99.66	0.68

*Average of five determinations.

The recovery technique was performed to judge the accuracy of the proposed method. For this, a known amount of the drug [10 or 20 mg] was added to the powder sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method. There was a high recovery of Nevirapine [100.18±0.67 or 101.08±0.82] indicating that the proposed procedure for the determination of Nevirapine in the tablet dosage forms is highly accurate. The results of recovery studies are expressed in Table 2.The proposed

found to be less than 0.68%.Also the inter day variation [3 days and five injections] was found to less than 0.86 %. The results of precision studies are expressed in Tables 3 & 4. Thus, the results showed that the proposed reversed phase HPLC method is highly reproducible.

CONCLUSION

The proposed HPLC method was found to be highly accurate, sensitive, and precise. Therefore this method can be applied for the routine qualify control

Table 4 Results of Inter day precision studies

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	200	198.84	99.42	0.86

*Average of five determinations.

analysis of Nevirapine in its tablet dosage forms.

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