A Reversed Phase High Performance Liquid Chromatographic Method for Estimation of Nevirapine in Tablets

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A reversed phase HPLC method is described for the determination of nevirapine in tablet dosage forms. Chromatography was carried out on an ODS column using a mixture of methanol and water (50:50 v/v) as the mobile phase at a flow rate of 0.9 ml/min. Cefixime was used as an internal standard and the detection was done at 230 nm. The retention time of the drug was 6.69 min. The method produced linear responses in the concentration range of 0.5 to 60 μg/ml of nevirapine. The method was found to be applicable for analysis of the drug in tablets. The results of the analysis were validated statistically.

Nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6H–dipyrido-[3,2-b:2',3'-e][1,4]-diazepin-6-one) is an orally active antiHIV drug[1]. The drug is a non-nucleoside reverse transcriptase inhibitor, which selectively inhibits HIV-1 replication. A few HPLC methods were reported earlier for the estimation of nevirapine in human plasma[2-8]. The authors now propose a new, rapid, sensitive and validated HPLC method for the estimation of nevirapine in tablet dosage forms.

A Shimadzu LC-10AT isocratic High Pressure Liquid Chromatographic instrument provided with an ODS C-18 reversed phase column (250×4.6 mm ID), 25 ml Hamilton injecting syringe, an SPD-10A UV/Vis detector and supported by a Windows based single channel software was employed in the study. HPLC grade methanol (E. Merck India Ltd. Mumbai) and triple distilled water were used for preparing the mobile phase. A freshly prepared 50:50 v/v mixture of methanol and water, which was filtered through a 0.45 μm membrane filter and sonicated, was used as the mobile phase. The flow rate of the mobile phase was maintained at 0.9 ml/min and the run time was set to 10 min. The column temperature was maintained at 25±1°. The detection was done at a wavelength of 230 nm.

A pure sample of nevirapine was used as a reference standard in the study. About 50 mg of nevirapine was weighed accurately and transferred into a 50 ml volumetric flask and dissolved in 25 ml of the mobile phase. The solution was sonicated for 15 min and then the volume made up with a further quantity of the mobile phase to get a 1 mg/ml solution. Subsequent dilutions of this solution ranging from 0.1 to 60 μg/ml were made in 10 ml volumetric flasks after addition of 0.5 ml cefixime solution (50 μg/ml) as an internal standard to each dilution. Twenty microlitres of the solution was injected each time into the column at a flow rate of 0.9 ml/min. Each of the dilutions was injected five times into the column and the corresponding chromatograms were obtained. From these chromatograms, the retention times and areas under the peaks of the drug and the internal standard were noted. Using the area values, the mean ratio of peak area of the drug to that of the internal standard for each dilution was calculated. The regression of the drug concentrations over these ratios was computed. This regression equation was later used to estimate the amount of nevirapine in pharmaceutical dosage forms. To check the intra-day and inter-day variation in the drug content determination, solutions containing 5, 10 and 15 μg/ml of nevirapine were subjected to the proposed HPLC analysis. To carry out the recovery studies, further known amounts of nevirapine were added to the preanalyzed drug solutions and then the samples were analyzed by the proposed HPLC method.

For testing the suitability of the proposed method to es-

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TABLE 1: ASSAY OF NEVIRAPINE IN TABLET DOSAGE FORMS

Brand name of the tablet formulation	Labelled amount (mg) of drug	Percent mean (±sd) amount (mg) found (n=5) by the	
		Proposed method	Reference method[8]
Nevimune	200	100.6±1.23	99.9±0.56
Nevivir	200	99.89±0.34	99.8±0.44

timate nevirapine in tablet formulations, two commercial brands of tablets (Nevimune of Cipla and Nevivir of Genix Laboratories) were chosen. For the testing, twenty tablets were weighed, powdered and an accurately weighed portion of this powder equivalent to 50 mg of nevirapine was transferred to a 50 ml volumetric flask containing 25 ml of the mobile phase. The contents of the flask were allowed to stand for 6 h with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 μm membrane filter. Appropriate volume of this filtrate was transferred into a 10 ml volumetric flask along with 0.5 ml of solution of cefixime (internal standard) and the volume made up with the mobile phase. This solution containing 10 μg/ml of nevirapine and 5 μg/ml of the internal standard was injected (20 μl) into the column. The mean peak area ratio of the drug to the internal standard of five such determinations was calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

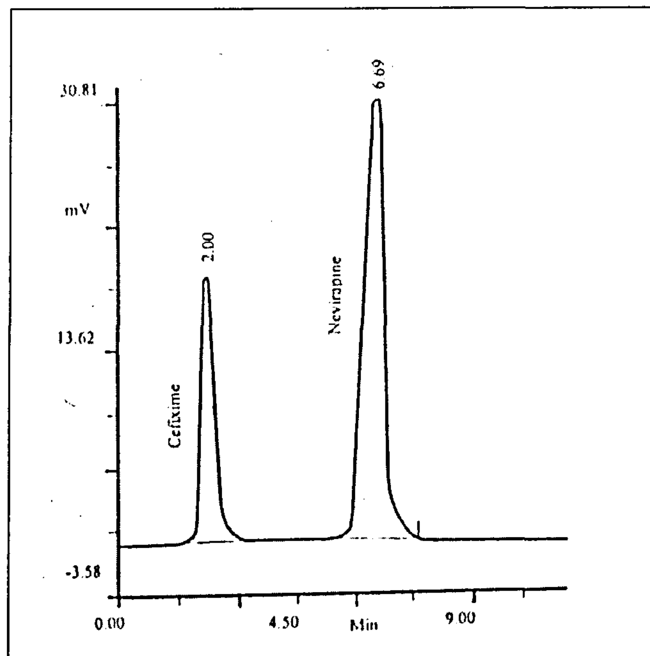

Fig. 1: Typical chromatogram of nevirapine

The present study was undertaken to develop a sensitive, precise and accurate HPLC method for the analysis of nevirapine in pharmaceutical dosage forms. A mixture of methanol and water in a 50:50 v/v proportion was found to be the most suitable of the various combinations of solvents tried. With this solvent system, the chromatographic peaks were better defined and resolved and almost free from tailing. Under the above-mentioned chromatographic conditions, the retention times obtained for nevirapine and cefixime were 6.69 and 2.00 min, respectively. A model chromatogram is shown in fig.1. A calibration of the proposed method indicated that the peak areas of both the drug and the internal standard were reproducible as indicated by a low percent coefficient of variation of 1.23. A good linear relationship (r=0.9998) was observed between the concentration of nevirapine and the respective ratio of peak areas. The corresponding regression curve was constructed by linear regression fitting and its mathematical expression was $y=1.9265x+0.3783$ (where y is the ratio of area under the curve of the drug to that of the internal standard and x is the corresponding concentration of nevirapine). The low coefficients of variation observed in the intra-day (0.48) and inter-day (0.53) variation study shows that the proposed HPLC method is highly precise. Recoveries ranging from 99.8 to 102.6 % of nevirapine from the pre-analyzed samples indicate the high accuracy of the method.

The drug content in the tablets was quantified by using the proposed analytical method. The percent mean amounts of nevirapine in two different brands of tablet dosage forms obtained by the proposed and reference methods are shown in Table 1. The absence of additional peaks in the chromatogram indicates non- interference of the common excipients used in the tablets. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of nevirapine in pharmaceutical dosage forms within a short analysis time.

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