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ESTIMATION OF NEVIRAPINE IN TABLETS BY HPLC

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ABSTRACT

A reserved phase HPLC method is described for the determination of nevirapine in pharmaceutical dosage forms. Chromatography was carried out on an ODS column using a mixture of acetonitrile and water (50:50 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Nimesulide was used as an internal standard and the detection was done at 230 nm using a UV detector. The retention time of the drug was 3.89 min. The method produced linear responses in the concentration range of 0.5 to 40 µg/mL of nevirapine. The method was found to be reproducible for analysis of the during in tablets.

Key words: Nevirapine, Estimation, Tablets, HPLC

INTRODUCTION

Nevirapine (11–cyclopropyl–5, 11–dihydro–4–methyl– 6H – dipyrido [3, 2–b; 2', 3'–] [1, 4] diazepin – 6 – one) is an orally active anti–HIV drug[1, 2] . This 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[3–8]. There are no reports of methods for its estimation in pharmaceutical dosage forms. Therefore, an attempt was made to develop a rapid, sensitive and validated HPLC method for the estimation of nevirapine. The utility of this method in determining the drug in commercial dosage forms was also demonstrated.

EXPERIMENTAL

Chromatographic condition : A Schimadzu LC–10AT isocratic High Pressure Liquid Chromatographic instrument provided with a SPD–10A UV–Vis detector, an ODS C–18 reversed phase column (4.6 mm I.D. x 25 cm), µL Hamilton injecting syringe and monitored by Windows based single channel software was employed in the study. HPLC grade acetonitrile (E. Merck India Ltd.) and triple distilled water were used for preparing the mobile phase. A freshly prepared 50:50 v/v mixture of acetonitrile and water was used as the mobile phase. Both

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acetonitrile and water were filtered through 0.4 µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1 mL/min. The column temperature was maintained at 25 ± 1°C. The detection was carried out at 230 nm. The column pressure was around 105 kgf/cm^2 during the experiment.

Drug and internal standard solutions : A pure sample of nevirapine procured from M/s. Hetero Drugs, Hyderabad, was used as 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 1 mg/mL solution. Subsequent dilutions of this solution ranging from 0.2 to 40 µg/mL were made in 10 mL volumetric flasks after addition of 0.5 mL nimesulide 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 1mL/min. Each of the dilutions was injected 5 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the areas under the peaks of the drug and the internal standard were noted. Using these 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 used to estimate the amount of nevirapine in pharmaceutical dosage forms.

Solutions containing 5 to 15 µg/mL of nevirapine were subjected to the proposed HPLC analysis to check the intra–day and inter–day variation of the method. The recovery studies were carried out by adding known amounts of nevirapine to the preanalyzed samples and then analyzing them by the proposed HPCL method.

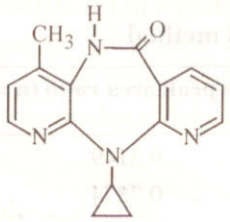
Estimation of nevirapine in tablet dosage forms

Two commercial brands of tablets (Nevimune of Cipla and Nevivir of Genix) were chosen for testing suitability of the proposed method to estimate nevirapine in tablet formulations. Twenty tablets were weighed and powdered. 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 hrs with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 µ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that the concentration of internal standard in each solution was 5 µg/mL. The contents of the flasks were made up to the volume with the mobile phase and mixed well. Each of these solutions (20 µL) was then injected 5 times into the column. The mean peak area ratio of the drug to the internal standard of 5 such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of nevirapine in pharmaceutical dosage forms. In order to effect analysis of the component peaks under isocratic conditions, mixtures of methanol or acetonitrile with water in different combinations were tested as the mobile phase on a C–18 stationary phase. A binary mixture of acetonitrile and water in 50:50 proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing with this system. Though the structure of nimesulide is not similar to nevirapine, it was chosen as the internal standard because it showed better peak shape and peak location compared to other potential internal standards such as nimodipine and nifedipine. Under the above mentioned chromatographic conditions, the retention times obtained for nevirapine and the internal standard were 3.89 and 8.17 min, respectively. A model chromatogram is shown in Fig. 1.

STRUCTURE OF NEVIRAPINE



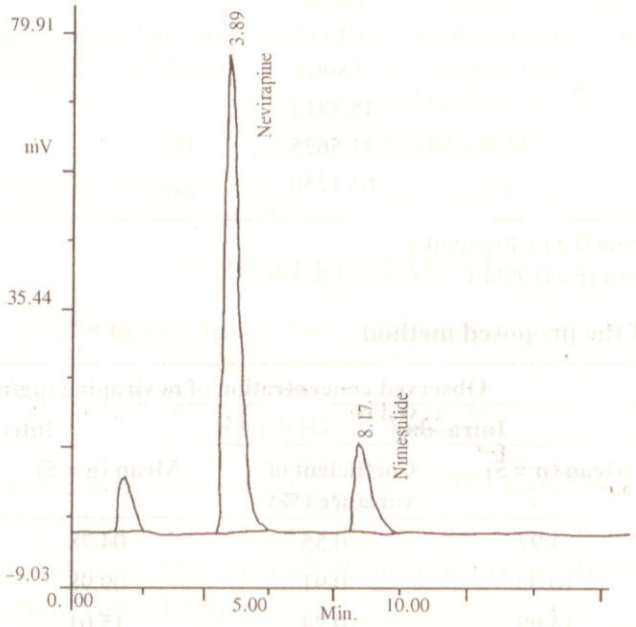


Fig. 1 Model chromatogram for nevirapine

Each of the samples was injected 5 times and the same retention times were observed in all cases. The ratios of peak area of nevirapine to peak area of internal standard for different concentrations set up as above were calculated and the average values for 5 such determinations are shown in Table 1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (1.01%). A good linear relationship (r = 0.9999) was observed between the concentration of nevirapine and the respective ratio of peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was y = – 0.05643 + 1.57920 x (Where y is ratio of area under the curve of the drug to that of the internal standard and x is the concentration of nevirapine). When nevirapine solutions containing 5, 10 and 15 µg/mL were analyzed by the proposed method for finding out intra and inter–day variations, a low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The amounts of nevirapine obtained from the preanalyzed samples containing known amounts of added drug are shown in Table 3. About 99.97% of nevirapine could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

Table 1. Calibration of the proposed method

Concentration of nevirapine (µg/mL)	Mean peak area ratio (n = 5)	Coefficient of variance (%)
0.2	0.3149	0.25
0.5	0.7874	0.03
1	1.5781	0.98
2	3.1497	0.65
5	7.8903	0.28
10	15.7812	1.01
20	31.5625	0.95
40	63.1250	0.73

Regression Equation (from 0.2 to 40 µg/mL)
y = – 0.05643 + 1.57920 x (r = 0.9998)

Table 2. Precision of the proposed method

Concentraion of nevirapine (µg/mL)	Observed concentration of nevirapine (µg/mL)			
	Intra–day		Inter–day	
	Mean (n = 5)	Coefficient of variance (%)	Mean (n = 5)	Coefficient of variance (%)
5	4.97	0.55	04.78	0.62
10	10.09	0.91	09.98	0.87
15	14.99	0.24	15.01	1.32

Table 3. Recovery data of nevirapine

Amount of drug added (μg) to solutions of pure drug/tablet formulation	Recovery from drug solution		Recovery from tablet formulation	
	Mean (± s.d.) amount (μg) found (n = 5)	Mean (± s.d.)% recovery (n = 5)	Mean (± s.d.) amount (μg) found (n = 5)	Mean (± s.d.)% recovery (n = 5)
2	2.08 ± 0.61	100.9 ± 0.35	2.011 ± 0.73	100.55 ± 0.88
4	4.052 ± 0.54	101 ± 0.78	3.999 ± 0.11	99.97 ± 1.53
10	9.997 ± 0.72	99.97 ± 0.55	10.024 ± 0.41	100.24 ± 0.78

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of nevirapine in two different brands of tablet dosage forms is shown in Table–4. The absence of additional peaks in the chromatogram indicates non–interference of the common excipients used in the tablets. The tablets were found to contain 99.89 to 99.93% of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of nevirapine in 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. The method was duly validated by evaluation of the required parameters.

Table 4. Assay of nevirapine tablet dosage forms

Brand name of the tablet	Labeled amount (mg) of drug	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d.) labeled amount (n = 5)
Nevimune	200	199.87 ± 0.24	99.93 ± 0.56
Nevivir	200	199.79 ± 0.85	99.89 ± 0.44

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