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Development and Validation of Reversed Phase HPLC Method for the Estimation of Nevirapine in Pure Form and in Pharmaceutical Dosage Forms

D. GOWRI SANKAR* and D.V.S.P. KUMAR

Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India

A simple, precise reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of nevirapine in bulk as well as in pharmaceutical dosage forms. The quantification was carried out using a BDS Hypersil C-18 column 250 mm × 4.6 mm i.d., 5 μm particle size in isocratic mode, with mobile phase comprising phosphate buffer and acetonitrile in the ratio of 75 : 25 (v/v). The flow rate was 1.5 mL /min and the detection was carried out at 220 nm. The retention time was 6.748 min. The method produced linear response in the concentration range of 60–130 μg/mL and the percentage recovery ranged from 98.15–99.01.

Key Words: RP-HPLC, Nevirapine tablets..

INTRODUCTION

Nevirapine is a non-nucleotide reverse transcriptase inhibitor. Chemically, it is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2′, 3′-e][1,4] diazepin-6-one. It is indicated in progressive or advanced HIV infection. Literature survey[1–6] indicates few HPLC methods are reported. In the present study, an attempt was made to develop a new simple, sensitive and fully validated method for the estimation of nevirapine in both bulk as well as in pharmaceutical dosage forms. It offers an alternative to the HPLC procedures available.

EXPERIMENTAL

A pure sample of nevirapine was received from Cipla Laboratories, Mumbai. HPLC grade acetonitrile (E. Merck, India), potassium dihydrogen phosphate of AR grade (Rankem Ltd.), potassium dihydroxide (Rankem), milli-Q water and samples containing nevimune (Cipla), Neve (Le Sante) were employed in the study.

An isocratic high performance liquid chromatograph using Waters 2695 separations module equipped with 600e controller pump and 776 auto sampler. Detection was carried out with a model 2487 dual λ absorbance detector. The chromatographic column used was a 250 × 4.6 mm hypersil BDS C-18 with 5 μm particles.

pH 7.5 buffer preparation: 3.24 g of potassium dihydrogen orthophosphate was diluted to 1 L with water and pH adjusted to 7.5 with potassium hydroxide.

Chromatographic conditions: Freshly prepared 75 : 25% (v/v) phosphate buffer and acetonitrile was used as the mobile phase. Phosphate buffer and acetonitrile were filtered through 0.45-μ membrane filter and degassed before use. The flow rate of the mobile phase was maintained at 1.5 mL /min. The column was maintained at ambient temperature. The detection was carried out at 220 nm. The injection volume was 10 μL and the run time was 15 min.

Procedure: The HPLC estimation was accomplished by external standard method. About 100 mg of pure sample of nevirapine was weighed accurately and transferred to a 100 mL volumetric flask and dissolved in 75 mL of the mobile phase. The solution was sonicated for 20 min and then the volume made up to volume with a further quantity of mobile phase to get 1 mg/mL solution. Six sets of solutions were prepared in mobile phase ranging from 60–130 μg/mL was in 10 mL volumetric flasks. The solutions prepared as above were filtered through 0.45 μm membrane filter and then 10 μL of filtrate was injected each time into the column at a flow rate of 1.5 mL /min. Each of the concentrations was injected five times into the chromatogram and the corresponding chromatograms were obtained. From these chromatograms, the retention time and mean peak area of the drug at each concentration were recorded. Evaluation of the drug was performed at 220 nm. The plot of peak area *vs.* the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was calculated using least squares methodology. This regression equation was later used to estimate the amount of nevirapine in pharmaceutical dosage forms.

Estimation of nevirapine in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate nevirapine in tablet formulations. For this, 20 tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 100 mg was taken in a 100 mL volumetric flask and 75 mL mobile phase was added. The solution was sonicated for complete solubility of the drug, made up to the mark with mobile phase and filtered through a 0.45-μ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to volume with the mobile phase and mixed well. Each of these solutions (10 μL) was then injected five times into the column. From the peak areas, the drug content in the tablets was quantified using the regression equation obtained from the pure sample.

Accuracy: The accuracy of the HPLC method was assessed by adding known amount (80, 100 120 μg/mL) of drug to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. Also a known amount of drug solution (80, 100, 120 μg/mL) was added to volumetric flask containing the sample of tablet formulation with known amount of drug. The drug was estimated as per the procedure described for the estimation of nevirapine in tablet formulations. In both cases, the recovery studies were replicated five times.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive, accurate and precise HPLC method for the analysis of nevirapine in bulk as well as in tablet dosage forms. For this, a binary mixture of phosphate buffer and acetonitrile in 75 : 25% (v/v) proportion was found to be the most suitable mobile phase as the chromatographic peaks obtained with the system were better defined and almost free from tailing. Under the above mentioned conditions, the retention time for nevirapine was 6.748 min. A typical chromatogram is shown in Fig. 1.

The peak areas from such different concentrations set up as above were calculated and are shown in Table-1. A good linear relationship ($r = 0.9999$) was observed between the concentration of nevirapine and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathemtical expres-

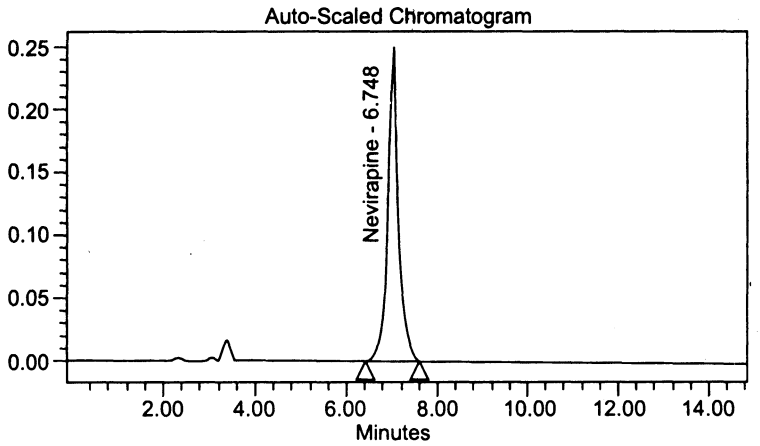

Fig. 1

sion was y = 23126.37x + 2265.4 (where y is the peak area and x is the concentration of nevirapine).

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (μg/mL)	Peak area*	% CV
60	1388548	1.90
80	1851704	1.42
100	2314886	0.98
120	2777812	1.59
140	3240836	0.82
150	3472330	1.67

*Mean of five determinations.

The present HPLC method was also validated for precision and accuracy. When a solution containing the drug was repeatedly injected, the coefficient of variation in the peak area was found to be less than 1%. This shows that the proposed HPLC method is highly reproducible. The results are shown in Table-2.

TABLE-2
PRECISION OF THE PROPOSED METHOD

Concentration of nevirapine (μg/mL)	Observed concentration of nevirapine (μg/mL)	
	Mean (n = 5)	Intra-day %CV
80	79.21	0.89
100	98.67	0.65
120	98.23	0.95

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of the drug with preanalyzed sample and contents were reanalyzed by the proposed method. High recoveries above 98% of nevirapine from the preanalyzed samples indicate the high accuracy of the proposed method (Table-3).

TABLE-3
RESULTS OF RECOVERY STUDY

Amount of drug added (μg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n = 5)	Mean % recovery	Mean amount found (n = 5)	Mean % recovery
80	78.52	98.15	79.67	99.58
100	99.4	99.40	98.90	98.90
120	118.82	99.01	119.17	99.30

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. A known amount of drug solution was added to sample of tablet dosage form and subjected to the estimation of drug by proposed method. The high recovery shows that the proposed procedure for tablet dosage form is highly accurate. The absence of additional peaks in the chromatogram indicates non-interference of common excipients used in the tablets.

TABLE-4
ASSAY OF NEVIRAPINE IN TABLET DOSAGE FORMS

Brand name	Labelled amount of drug (mg)	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d.) % labelled amount (n = 5)
Neve	200	199.93 ± 0.03	99.96 ± 0.05
Nevimune	200	198.92 ± 0.23	99.46 ± 0.12

It can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of nevirapine in tablet dosage forms.

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