QUANTITATIVE ESTIMATION OF NEVIRAPINE BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

A simple, accurate and precise HPTLC method has been developed and validated for the estimation of nevirapine from bulk drug and tablet formulations. The separation was achieved on TLC plates using appropriate solvent system. The spots so developed were densometrically scanned at 283 nm. The linearity of the method was found to be within the concentration range of 2.50µg/ml to 62.50µg/ml. The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of this method. The method was successfully applied for determination of drug in tablets, wherein no interference from tablet excipients was observed, indicating the specificity of the developed method. Thus the proposed method can be used successfully for routine analysis of nevirapine from capsule and tablet formulations.

Keywords: Nevirapine, HPTLC, validation, precise, accurate

INTRODUCTION

Nevirapine (Fig.1), 11-cyclopropyl-4-methyl-5, 11-dihydro-6*H*-dipyrido [3, 2-*b*: 2', 3'-*e*][1,4] diazepin-6-one is a reverse transcriptase (RT) inhibitor of human immunodeficiency virus type 1 (HIV-1)[1,2]. Nevirapine inhibits replication of HIV-1 by interfering with viral RNA-directed DNA polymerase (reverse transcriptase). It binds directly to herodimeric HIV-1 reverse transcriptase and exerts a virustatic effect by acting as a specific, noncompetitive HIV-1 reverse transcriptase inhibitor; it appears to inhibit viral RNA- and DNA-dependent DNA polymerase activities by disrupting the catalytic site of the enzyme [3].

Literature survey reveals that there are analytical methods available for determination of nevirapine from biological matrices [4-14], bulk drug and dosage forms [15-17], and analytical methods for determination of nevirapine with combination of other antiviral drugs [18-50] Literature survey further revealed that there were very few reported HPTLC method for the analysis of nevirapine. Thus, an appropriate analytical procedure for the quantitative determination of nevirapine from bulk drugs is of considerable importance.

Keeping this objective in mind an attempt has been made to develop and validate HPTLC method for the analysis of nevirapine which would be highly sensitive, having good resolution and reproducible. Various validation aspects of the analysis, accuracy, precision, recovery and the limits of detection and quantification etc., have been measured.

EXPERIMENTAL

Chemicals and Reagents

The working standard of nevirapine was procured from Cipla Ltd., India. HPLC grade ethyl acetate and methanol was purchased from Merck (Darmstadt, Germany). Deionised and ultra pure water used in all experiments was obtained from Milli-Q System (Millipore). Glacial acetic acid of AR (Analytical Reagent) grade was procured form S. D. Fine Chemicals (India).

Equipment

The HPTLC system employed in the method development and validation was Camag Linomat V applicator (Muttenz, Switzerland), a Camag twin trough chamber of appropriate size, Camag TLC scanner III, Wincats (version 1.2.2) software as data integrator and a Hamilton syringe (Switzerland) of 100μl capacity.

Preparation of Standard Solution

A stock solution of nevirapine (1mg/ml) was prepared in methanol. Standard solution was prepared by dilution of the stock solution with methanol to give solution in of 100μg/ml. Further dilutions were made with methanol to give solution in concentration range of 2.50μg/ml to 62.50μg/ml.

HPTLC

Prewashing of Plates

HPTLC was performed on 10 cm x 20 cm precoated silica gel GF_{254} pre-coated HPTLC plates from E.Merck (Darmstadt, Germany). The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack

has been opened. The non volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried and activated for 30 min at 110^0C, with the plates being placed between two sheets of glass to prevent deformation of the aluminium during heating.

Procedure

A methanolic solution of nevirapine (1mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 100 μg/ml. Different concentration of nevirapine in concentration range of 2.50μg/ml to 62.50μg/ml were applied on plates as 6 mm bands, 6 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100 μl Hamilton syringe. A methanol blank was applied to parallel track. After drying of bands, the plate was placed in one of the troughs of Camag twin trough glass chamber. The mobile phase, ethyl acetate: glacial acetic acid (10:0.1 %v/v) was poured into the second trough and the plates were left to equilibrate in the chamber for 10 min at $25 \pm 2\ ^0$C. The plate was then moved to the second trough containing the mobile phase and developed to a distance 90 mm. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 283 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. The representative densitogram obtained by HPTLC of nevirapine at various concentrations are shown in Fig.3. This method was followed for all quantitative analysis. Wincats software (version 1.2.2) was used for data acquisition and processing of the plate. The scanning speed was 20 mm s^{-1} , the offset was 10 %, and the sensitivity (SPAN) was optimized to 20 min. Peak height and peak area were integrated for the entire track. Calibration curve was

established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa. The reproducibility of this method was ascertained by repeating the experiment six times.

Method Validation

The method was validated for specificity, accuracy, precision by use of calibration standards of nevirapine. Limit of detection and limit of quantitation were determined by the visual method, by spotting different concentration of nevirapine. The lowest concentration that could be detected for three replicate spots was regarded as the limit of detection. The lowest concentration for which the RSD [%] of six replicate spots was less than 5 % was regarded as the limit of quantitation. LOD was calculated using formula LOD=3.3xσ/S, where σ is residual standard deviation of regression line and S is the slope of corresponding line. Accuracy was determined by measurement of the recovery of nevirapine standard added at three different levels, each being analyzed as described for the assay. Intraday and interday precision was determined by applying 7.50µg/ml, 25.00µg/ml and 62.50µg/ml standard nevirapine. After development and densitometric scanning of the plates the peak-area response was measured and precision was calculated as RSD [%]. The method was validated as per ICH guidelines.[51]

Application of the validated method

To determine the content of the drug in solid dosage form, 20 tablets of nevirapine (200 mg) were accurately weighed, their average weight was calculated. Powder equivalent to 200 mg of the drug was dissolved in 100 ml methanol in a volumetric flask and sonicated for 20 min. (Solution A ~ 2000 µg/ml). Further dilutions were made with mobile phase as follows: 1 ml of Solution A was diluted to 100ml in a volumetric flask (Solution B ~ 20 µg/ml). 1ml of Solution

B was diluted to 10ml in a volumetric flask (Solution C ~ 2 µg/ml). This Solution C was spotted

(40µl) on to the HPTLC plate. The average area of 6 such spots was taken for calculation.

Concentration of nevirapine was calculated from the standard graph, which was plotted using the

area of known concentration (2.50-62.50µg/ml) of nevirapine standard. The results are

summarized in Table 1.

Label Claim	200 mg
Amount found ±SD (n=6)	201.11 ± 0.522 mg
% Label Claim	100.56 %
% RSD	0.259

n= no. of repetitions

Table 1 Assay of Nevirapine in Tablet

Linearity and range	Nevirapine
Range (µg/ml)	2.50 - 62.50
r^2	0.9959
Slope	396.88

Table 2 Linearity and Range

Precision			% Recovery	
Actual Concentration (µg/ml)	**Measured Concentration (µg/ml) ± S.D.; % R.S.D.**			
	Intra-day	Inter-day	Intra-day	Inter-day
7.50	7.56 ± 0.22; 2.96	7.49 ± 0.20; 2.72	100.76	99.84
25.00	24.65 ± 0.519; 2.06	24.84 ± 0.50; 2.01	98.61	99.38
62.50	62.54 ± 0.77; 1.23	62.38 ± 0.70; 1.12	100.07	99.81

Table 3 Precision and Recovery data
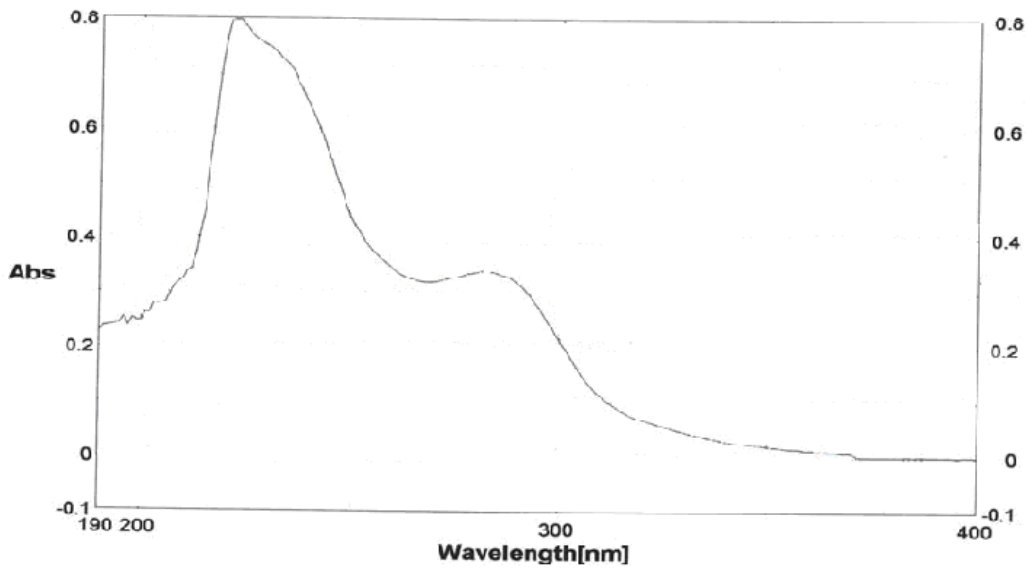

Fig.1Chemical Structure of Nevirapine

Fig. 2 A typical Absorption Spectrum of Nevirapine Scanned at 200-400 nm

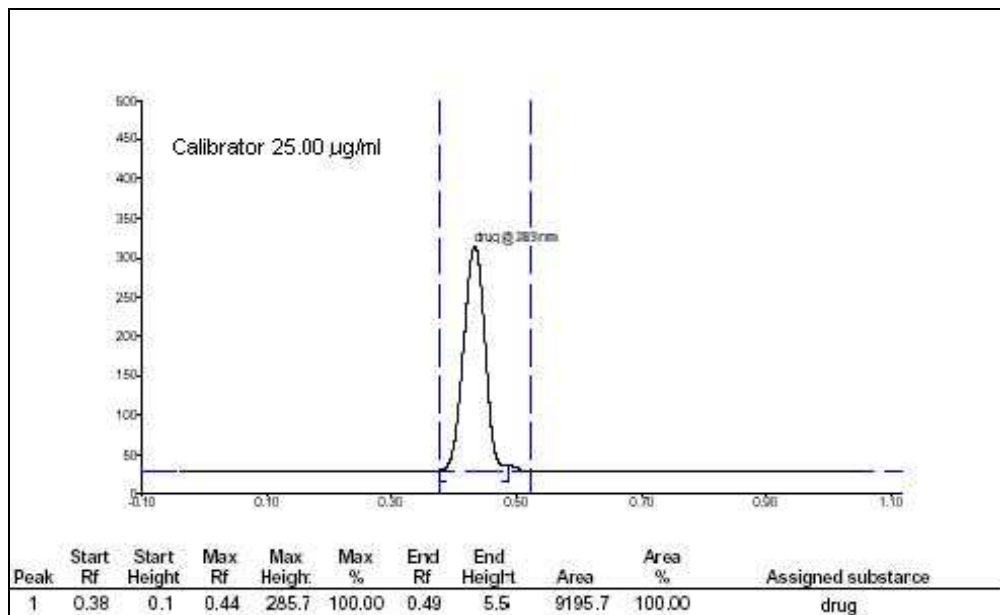| Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.38	0.1	0.44	285.7	100.00	0.49	5.5	9195.7	100.00	drug

Fig. 3 Representative Chromatogram of Standard 25µg/ml Nevirapine at 283 nm
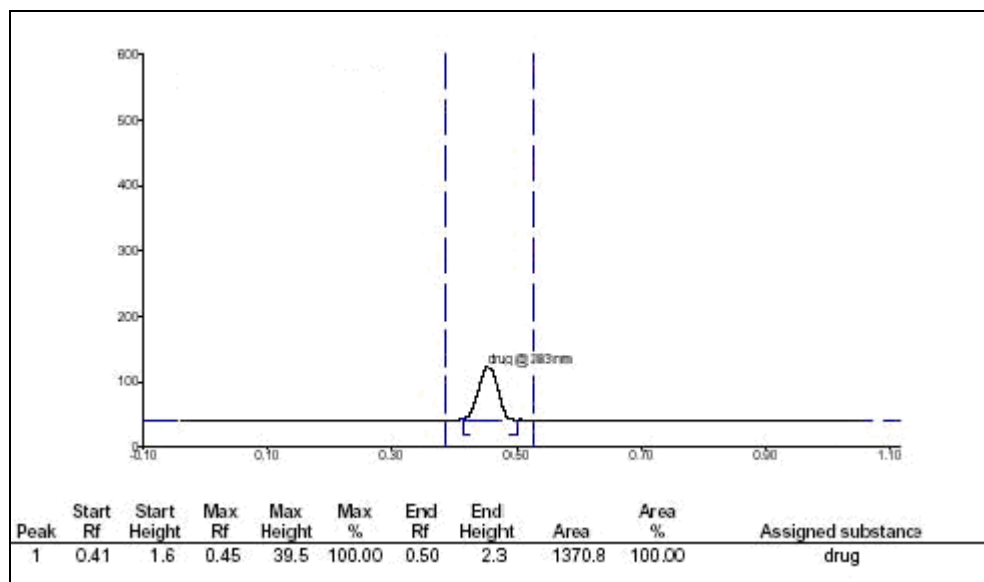
Fig. 4 Representative Chromatogram of Formulation of Nevirapine at 283 nm

RESULTS AND DISCUSSION

The solvent chosen for HPTLC must take into account the chemical nature and polarity of the drug molecule. Nevirapine is practically insoluble in water, soluble in dichloromethane, dimethylsulphoxide and dimethylformamide, slightly soluble in methanol. Calibrator solutions were prepared in methanol. Solubility increases at pH <3. Initially acetonitrile: water (1:1) was used as the solvent system, but it was observed that the spot did not travel at all and RF value was found to be negligible. Solvent system comprising of acetonitrile: water (2:1), methanol: water (2:1) were tried but again RF value was found to be negligible. Thus after much trials solvent system comprising of ethyl acetate gave good separation and there was no interference

around the drug RF. Slight tailing was observed which was removed when 0.1% glacial acetic acid was added in the solvent system Thus after much experiment, the optimized system was developed which consisted of ethyl acetate: glacial acetic acid (10:0.1 %v/v). The optimized system gave a good separation and resolution of nevirapine.

The plate material employed was silica gel 60 GF_{254}. Scanning of nevirapine on HPTLC plate showed UV max at 283 nm (Fig.2) hence quantitation was carried out at 283 nm using Camag TLC scanner III.

The method developed was validated for limit of detection (LOD) and limit of quantitation (LOQ) in order to determine and ensure sensitivity of the developed method. The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.84 and 2.5μg per spot respectively. The method was found to be linear over the range 2.50 - 62.50μg per spot with coefficient of regression 0.9959. (Table 2) Intra-day and inter-day precision studies showed a % RSD was less than 5.00%, indicating the method was precise. The accuracy values obtained, in the range 98.61 – 100.76 % for drug are indicative of excellent accuracy and recovery. There was no evidence of peaks or any other interfering co eluting peaks at the RF of standard (0.44). This indicates the method is specific. Stability studies were carried out for standard. It was found to be stable in sample solution, prior to development and after development.

The developed method was then validated and successfully applied for quantitation of nevirapine from the formulation. To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level, to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were

performed and the results obtained are shown in Table 3. The results of recovery studies were within the specified limits of ICH guidelines. Lower values of %RSD reflect the accuracy of the method. Precision, expressed in terms of %RSD was determined in terms of intra-day and inter-day precisions, analyzing the drug at three different concentrations, determining each concentration thrice. The sample solutions were analyzed using the method for 3 consecutive days, repeating the process twice a day at different period. The results obtained are summarized in Table 3 and reflect high degree of precision. Two different analysts performed assay on marketed tablets of the drug, in similar operational and environmental conditions, using the developed method to determine its ruggedness.

`   The optimized solvent system yielded a symmetrical peak for the drug with RF 0.44 (Fig.3). A typical absorbance spectrum of the drug is shown in Fig.2. The peak of the drug from tablets (Fig.4) was identified by comparing the RF and also comparing its absorbance spectrum with that obtained with the standard drug.

CONCLUSION

The developed and validated HPTLC method reported here is rapid, simple, accurate, sensitive and specific. The method was also successfully used for quantitative estimation and analysis of nevirapine from formulation. Thus the reported method is of considerable importance and has great industrial applicability for quality control and analysis of nevirapine from bulk drug and formulations.

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