RP-HPLC Method for the Estimation of Nelfinavir Mesylate in Tablet Dosage Form

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Abstract: A reverse phase HPLC method is described for the determination of Nelfinavir Mesylate in tablet dosage form. Chromatography was carried on an ODS column using a mixture of acetonitrile and phosphate buffer pH 6 (90:10 v/v) as the mobile phase at a flow rate of 1.2 mL/min with detection at 230 nm. The retention time of the drug was 6.68 min. The detector response was linear in the concentration of 1-20 mcg/mL. The limit of detection and limit of quantification was 1.0 and 10.0 mcg/ mL respectively. The percentage assay of Nelfinavir Mesylate was 99.77 %. The method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, fast, accurate and precise and hence can be applied for routine quality control of Nelfinavir Mesylate in bulk and tablet dosage form.

Keywords: Nelfinavir Mesylate, RP-HPLC, Estimation, and Tablets.

Introduction

Nelfinavir Mesylate¹ is a novel HIV-1 protease inhibitor; with a chemical name (3S, 4aS, 8aS)-N- (1,1-Dimethylethyl) decahydro-2- [(2R, 3R)-2-hydroxy-3- [(3-hydroxy-2-methyl benzoyl) amino]-4-(phenylthio) butyl]-3-isoquinolinecarboxamide methanesulfonate .It is an antiretroviral drug that acts by binding reversibly to HIV protease thereby preventing cleavage of the viral precursor polypeptides. It is official in Martindale²-The Extra pharmacopoeia. Literature survey reveals many Chromatographic methods³⁻¹⁰ for the determination of Nelfinavir in biological fluids and in combination with other antivirals and few Spectrophotometric methods¹¹⁻¹⁴ only. No method so far has been reported for the estimation of Nelfinavir Mesylate from pharmaceutical dosage form. The present paper aims

at reporting an isocratic RP-HPLC method for the estimation of Nelfinavir Mesylate in tablet dosage form.

Experimental

Nelfinavir Mesylate was obtained as a gift sample from Aurobindo Pharma Ltd, Hyderabad. Acetonitrile HPLC grade, sodium hydroxide AR grade, potassium hydrogen orthophosphate AR grade of Rankem ltd. Water HPLC grade of Milli-Q were used.

Instrument

High Performance Liquid Chromatograph, Waters Alliance separation module 2695, equipped with automatic injector with injection volume 100 μ L, Ultra-violet Visible detector Waters 2487 Dual alpha absorbance detector with Empower software.

Chromatographic conditions

Chromatographic separations were achieved using a Inertsil ODS C18 (250X4.6 mm, 5 μ) analytical column. The mobile phase consisting of acetonitrile and phosphate buffer pH 6 (90:10 v/v) was passed through 0.45 μ m membrane filter and degassed by ultrasonication. The flow rate was maintained at 1.2 mL /min and the measurements were made at 230 nm. The column and the HPLC system were kept in ambient temperature.

Preparation of Mobile phase: Phosphate buffer pH 6 was prepared by dissolving 1.36 gms of potassium hydrogen orthophosphate in 1000 mL of water and by adjusting the pH to 6 with 0.1 N Sodium Hydroxide.

Preparation of Standard Stock solution: Accurately weighed 50 mg of Nelfinavir Mesylate standard was taken in 50 ml volumetric flask. This was dissolved in 25 mL of mobile phase and sonicated for 5 mins, and then diluted to 50 mL with the mobile phase to get 1mg/ mL standard stock solution.

Working Standard solution: 5 mL of the above stock solution was taken in 50 ml volumetric flask and thereafter made upto 50 mL with mobile phase to get a concentration of 100 μ g/mL.

Preparation of Sample solution: Twenty tablets (Nelfin, Genix Pharma) were weighed accurately and finely powdered. The powder equivalent to 100 mg was taken in 100 mL volumetric flask. This was dissolved in 75 mL mobile phase and sonicated for 15 mins with internal shaking. Then the volume was finally made to 100mL. The above solution was centrifuged at 3000 rpm for 5 mins to get a clear solution. Then pipetted out 5 mL of clear supernatant liquid into 50 mL volumetric flask and made up the volume with mobile phase to get a concentration of 100 μ g/mL.

Linearity: Several aliquots of standard stock solutions (0.1,0.25,0.5,1.0,1.5 and 2.0) mL (1 mL=100 μ g/mL) of Nelfinavir Mesylate were taken in different 10 mL volumetric flask and diluted up to the mark with mobile phase. Evaluation was performed with Ultra-Violet Dual alpha absorbance detector at 230 nm. Peak area was recorded for all the peaks and a Calibration graph was obtained by plotting peak area versus concentration of Nelfinavir Mesylate (Figure 2). The plot of peak area of each sample against respective concentration of Nelfinavir Mesylate was found to be linear in the range of 1.0 – 20.0 μ g/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective slope (m), intercept (b), standard deviation and correlation coefficient are given in Table 1.

Table 1. Linear Regression Data for Calibration curves.

Drug	Nelfinavir Mesylate
Concentration range (mcg/mL)	1 – 20
Slope (m)	9940.18
Intercept (b)	6657.9
Correlation coefficient	0.9999
Variance	0.011
% RSD	0.2

Assay: 10 μ L of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 6.68 mins. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Table 2. Results of HPLC assay and Recovery studies

Sample	Amount claim (mg/tablet)	Amount found (mg/tablet)	% Recovery*
1.	625	618.5	99.65
2.	625	620.5	99.29
3.	625	626.3	100.27
4.	625	625.3	99.97
5.	625	627.5	100.45
		Mean = 623.6	Mean = 99.92

*Average of three different concentration levels.

Recovery Studies: Accuracy was determined by recovery studies of Nelfinavir Mesylate, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

Results and Discussion

As per the USP-XXIV system suitability tests were carried out on freshly prepared standard stock solution of Nelfinavir Mesylate. Parameters that were studied to evaluate the suitability of the system are given in Table 3.

Table 3. Validation Summary

Validation Parameter	Results
<u>System Suitability</u>	
Theoretical Plates (N)	7088
Linearity range (mcg/mL)	10-200
Tailing factor	1.1
Retention time in minutes	6.68
LOD (mcg/mL)	1.0
LOQ (mcg/mL)	10.0

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) for Nelfinavir Mesylate were found to be 1.0 and 10.0 μ g/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

From the typical chromatogram of Nelfinavir Mesylate as shown in Figure 1, it was found that the retention time was 6.68 min. A mixture of acetonitrile and pH buffer 6 in a ratio of 90:10 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship ($r=0.9999$) was observed between the concentration range of 1.0-20.0 mcg/mL. The assay of Nelfinavir Mesylate tablets was found to be 99.77%. From the recovery studies it was found that about 99.92 % of Nelfinavir Mesylate was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of Nelfinavir Mesylate within a short analysis time.

Figure 1. Typical Chromatogram of Nelfinavir Mesylate by HPLC

Figure 2. Calibration curve of Nelfinavir Mesylate by HPLC

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