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VALIDATED REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ESTIMATION OF NELFINAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Nelfinavir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile and Water (15:85% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 218nm. The retention time of the Nelfinavir was 5.430 ±0.02min. The method produce linear responses in the concentration range of 10-50ppm of Nelfinavir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Nelfinavir, RP-HPLC, validation.*

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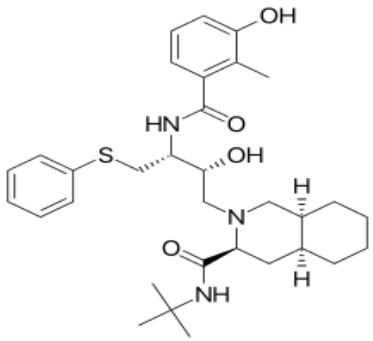
INTRODUCTION:

Nelfinavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Nelfinavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs.

Chemical structure of Nelfinavir



MATERIALS AND METHODS:

Chromatographic conditions

A perominence isocratic HPLC system (waters 2695 HPLC with auto sampler and PDA Detector) column Zorbax C18 (4.6×250mm 5µ). A 10µL Rheodyne injection syringe was used for sample injection. HPLC grade Water: Acetonitrile were used for the preparing the mobile phase. A freshly prepared Water: Acetonitrile (85:15% v/v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1mL/min., column temperature was maintained at room temperature and the detection of the drug was carried out at 218nm.

Preparation of mobile phase:

Accurately measured 850ml (85%) of HPLC Water and 150ml (15%) of HPLC Acetonitrile in to a 1000ml of volumetric flask and degassed in a digital ultrasonicator for 10 minutes.

Diluent preparation:

The Mobile phase was used as the diluent.

Standard solution preparation:

Accurately weigh and transfer 10 mg of Nelfinavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Nelfinavir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Sample solution preparation:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Nelfinavir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of Nelfinavir above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method validation

Linearity:

The linearity of the method was demonstrated over the concentration range of 10-50 ppm of the Nelfinavir Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Nelfinavir was constructed by plotting peak area versus applied concentration of Nelfinavir.. A typical chromatogram is shown in Fig 1. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in fig: 2. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table: 1 and their calibration parameters were shown in Table: 2.

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and% RSD were calculated and present in Table 4 . The chromatogram was shown in Fig 3. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peak and %RSD were calculated shown in Table 5. From the data obtained, the developed method was found to be precise.

Accuracy

A study of recovery of Nelfinavir from spiked placebo was conducted at three different spike levels i.e.50%, 100% and 150% samples were prepared with Nelfinavir raw material equivalent to about the target initial concentration of Nelfinavir. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table 4. The mean recoveries of Nelfinavir spiked were found to be in the range of 99.5%

LOD and LOQ:

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ (Table no.3)

System suitability

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30µg/ml. The results given in Table9 were within acceptable limits

Table: 2 Characteristic parameters of Nelfinavir for the proposed RP-HPLC method

Parameters	RP-HPLC
Calibration range (µg/ml)	of Nelfinavir
Detection wavelength	218
Mobile phase (Water: Methanol)	Water: Methanol 55:45% v/v
Retention time	5.430
Regression equation(Y*)	Y = mx + c
Slope (m)	17178
Intercept (c)	4318
Correlation coefficient (r^2)	0.999
Intraday precision (%RSD*) Interday precision (% RSD*)	0.42 0.73
Limit of detection (mcg/ ml)	2.2µg/ml
Limit of quantitaion(mcg/ml)	6.8µg/ml

Table 3. Precision results for Nelfinavir

Sl no	Intraday precision (area)	Interday precision (area)
1	516091	518081
2	518221	518221
3	519536	514561
4	519881	515381
5	519895	514561
6	522826	507837
Mean	519408.3	514773.7
Std Dev	2216.82	3778.868
% RSD	0.42	0.73

Table 4. Accuracy results for Nelfinavir

Sample No	Spike level	Amount (ppm) found	Amount (ppm) added	% Recovery	%Mean recovery
1	50%	14.9	15	99.3%	99.5%
2	100%	29.87	30	99.5%	
3	150%	44.89	45	99.8%	

Table: 5 system suitability studies of Nelfinavir by RP-HPLC method

Property	Nelfinavir Values	Required limits
Retention time (R_t)	5.430	RSD ≤ 1%
Theoretical plates (N)	9118	N > 2000
Tailing factor	1.03	T≤ 2

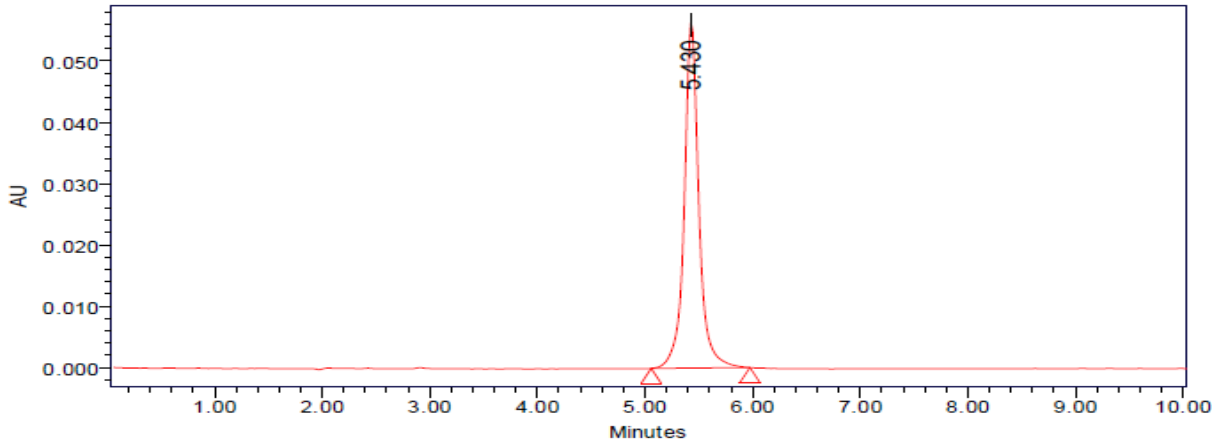


Fig: 1. Chromatogram of Nelfinavir at 218nm

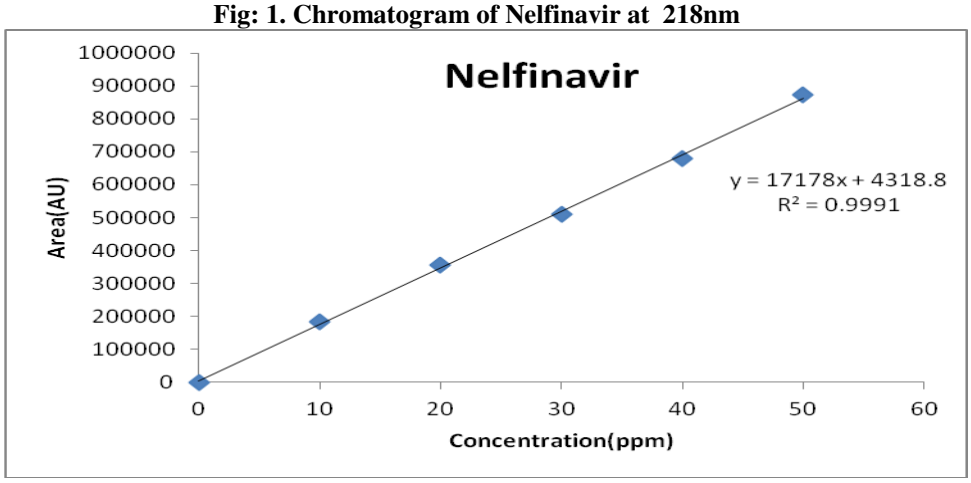


Fig:2. Calibration curve of Nelfinavir

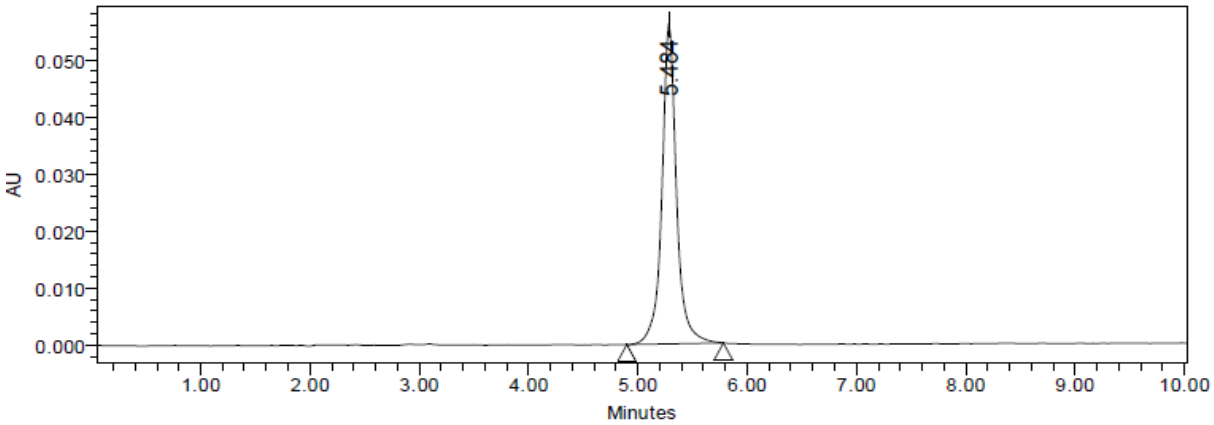


Fig:3. Chromatogram of precision Nelfinavir

RESULTS AND DISCUSSION:

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Nelfinavir bulk dug and pharmaceutical dosage form by using the most commonly employed Zorbax C18 column with PDA-detection.

The run time was set at 10 min and the retention time for Nelfinavir was 5.430 respectively. Each sample was injected 5 times and the retention times were same. When the concentrations of Nelfinavir and its respective peak areas were subjected to

regression analysis by least squares method, a good linear relationship (r^2=0.999) was observed between the concentration of Nelfinavir and the respective peak areas in the range 10-50 µg /ml of The regression equation was used to estimate the amount of Nelfinavir either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table: 2.

To analyse tablet formulations, RP-HPLC method has been developed. Nelfinavir tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries found in the range of 99.5% No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Nelfinavir in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Nelfinavir was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water.

Water: Acetonitrile (85:15% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Nelfinavir in bulk drug and in Pharmaceutical dosage forms.

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