Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (1):135-141
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Development and validation of reverse phase liquid chromatographic methods for the determination of Nelfinavir mesylate in tablet form

Pravin. B. Cholke*, A. S. Jadhav, S. K. Ingale, S. Z. Chemate, M. A. Raskar

P.D.V.V.P.F's College of Pharmacy, Vilad Ghat, Ahmednagar

ABSTRACT

A RP- 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 methanol and water (80:20 v/v) instead 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, Methanol and VIRACEPT® Tablets.

INTRODUCTION

Nelfinavir Mesylate1 is a novel HIV-1 protease inhibitor; with a chemical name (3S, 4aS,8aS)-N- (1,1-Dimethylethyl) dehydro-2- [(2R,3R)-2-hydroxy-3- [(3-hydroxy-2-methyl benzoyl) amino]–4-(phenylthio) butyl]-3 isoquinoline carboxamide methanesulfonate (Fig.1) .It is an antiretroviral drug that acts by binding reversibly to HIV protease thereby preventing cleavage of the viral precursor polyproteins (Fig.2). It is official in Martindale the Extra pharmacopoeia. Literature survey reveals many Chromatographic methods for the determination of Nelfinavir in biological fluids and in combination with other antiviral and few Spectrophotometric methods only. No method so far has been reported for the estimation of Nelfinavir Mesylate from pharmaceutical dosage form. The present paper aims on reporting an isocratic RP-HPLC method for the estimation of Nelfinavir Mesylate in tablet dosage form using methanol and water[1, 2, 3].

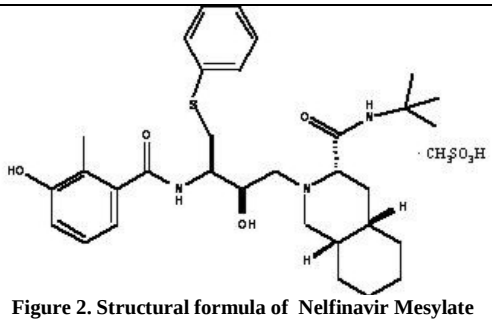

Figure 2. Structural formula of Nelfinavir Mesylate

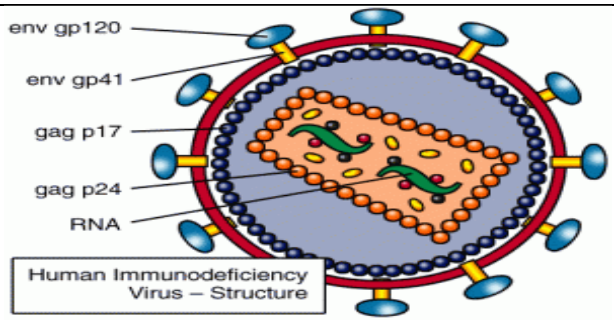

Figure 1. Structure of HIV

MATERIALS AND METHODS

Nelfinavir Mesylate pure drug was obtained as a gift sample from Aurobindo Pharma Ltd, Hyderabad (India). VIRACEPT® (250mg) tablets were purchased from Civil Hospital (A.R.T center) Ahmednagar. All the reagents in this assay along with Methanol and Water were of HPLC grade and the solution were prepared using preanalysed double distilled water.

Apparatus:
High Performance Liquid Chromatograph, Jasco LC-Net II/ADC module, equipped with syringe injector with injection volume 100 µL, Intelligent Ultra-violet Visible detector Jasco VU-2075 plus, Jasco PU-2080 plus Intelligent HPLC Pump with Empower software.

Chromatographic conditions: [4]
Chromatographic separations were achieved using an Inertsil ODS C18 (250X4.6 mm, 5µ) analytical column. The mobile phase consisting of methanol and water (80:20 v/v) was passed through 0.45 µm 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: Mobile phase was prepared by dissolving 80 ml of methanol and 20ml of water (80:20v/v) in 100ml volumetric flask.

Preparation Standard Stock Solution: [5]
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 up to 50 mL with mobile phase to get a concentration of 100 µg/mL

Preparation of Sample solution: Twenty tablets (VIRACEPT®) 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 pippetted 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 µg/mL

Preparation of Placebo solution: [7, 8, 9]
Placebo solution of Nelfinavir Mesylate was prepared by calculation of placebo content in each tablet

Placebo content = Average net content of tablet- weight of API per tablet.

Make same dilution as of Standard Stock Solution.

Determination of absorbance maximum and Preparation of calibration curve:
Several aliquots of standard stock solutions (0.1, 0.25, 0.5, 1.0, 1.5 and 2.0) mL (1 mL=100 µg/mL) of Nelfinavir Mesylate were taken in different 10 mL volumetric flask and diluted up to the mark with mobile phase[11]. Evaluation was performed with Jasco Spectrophotometer, Model-V-630 (Japan) 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 3). 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 µg/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.

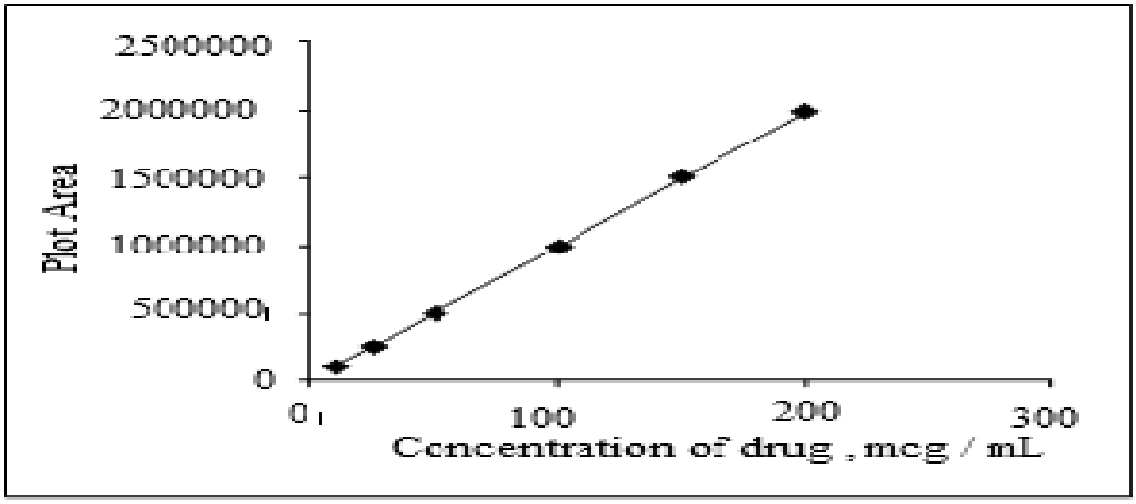

Figure 3 Calibration curve of Nelfinavir Mesylate by HPLC

Analysis of Marketed Tablet Formulation:
Assay: 10 µL 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[12]. The data are presented in Table 2.

Table 1. Linear Regression Data for Calibration curves.

Sr. no.	Parameters	Values
1	Absorption maxima, nm	230 nm
2	Beer's law limit, µg/ml	1-20
3	Molar absorptivity, 1 mole-1/cm-1	2.21×10-4
4	Coefficient of correlation(f)	0.9999
5	Regression equation	Y=0.053x+0.00
6	Slope(b)	0.9940
7	Intercept(a)	0.6657 ± 0.001215
8	%RSD	0.2
9	Solvent	Methanol and water
10	LOD and LOQ	0.4ppm and 1ppm

Table 2. Results of HPLC assay and Recovery studies

Sample	Amount claim (mg/tablet)	Amount found (mg/tablet)	% Recovery*
1	250	237.6	99.65
2	250	241	99.29
3	250	252.6	100.27
4	250	250.6	99.97
5	250	255	100.45
		Mean =247.36	Mean = 99.92

** Average of three different concentration levels*

Table 3. Validation Summary

Validation Parameter	Results
System Suitability	
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

Method validation:

Accuracy (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.

System Suitability: [13]

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.

Limit of Detection (LOD) and Limit of Quantification (LOQ):[14]
The limit of detection (LOD) and limit of quantification (LOQ) for Nelfinavir Mesylate were found to be 1.0 and 10.0 µg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

Specifity: [15]
Specifity was validated using stress condition such as acid (0.1N HCL), base (0.1N NAOH) and Oxidant (3%H2O2)

The forced degradation of Nelfinavir Mesylate API, Nelfinavir Mesylate standard, Nelfinavir Mesylate tablet sample was tested. The Specifity was validated with no interference of other peaks at Rt of main.

Ruggedness: [16]
Ruggedness was validated by using-different analyst, different column and different system were used and all the parameters for chromatography remain same. The five replicate standards were injected and then samples were run. The ruggedness will be established on the basis of %RSD which should not be more than 2% for all six ruggedness sets and six precision sets of sample preparation. Six samples were prepared and injected for establishment.

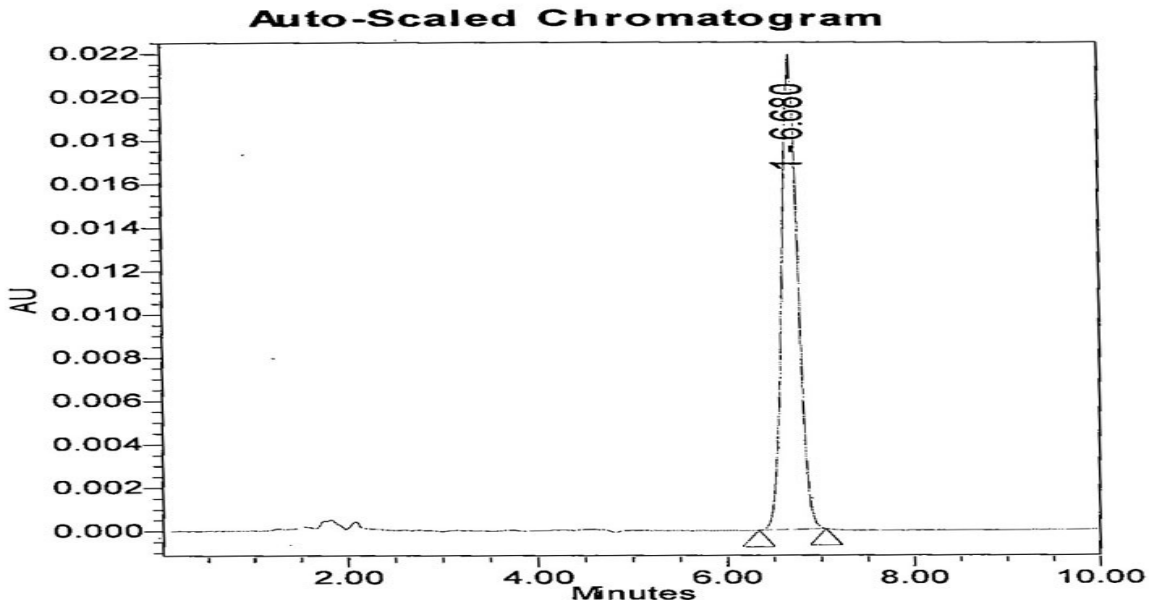

Figure 4 Typical Chromatogram of Nelfinavir Mesylate by HPLC

Precision: [6]
The Precision of the proposed method was ascertained by actual determination of ten replicates of fixed concentration of the drugs within the Beer's range and finding out the absorbance (AU) by the proposed method. From this absorbance, mean, SD, %RSD was calculated. The readings were shown in table no.4.

Table no.4 Precision reading of Nelfinavir Mesylate

Sr. no.	Concentration (µg/ml)	AU	Statistical analysis
1	20	1.064	Mean 1.060
2	20	1.051	S.D. 0.975
3	20	1.061	%RSD.91.98.
4	20	1.063	
5	20	1.060	
6	20	1.064	
7	20	1.065	
8	20	1.056	
9	20	1.066	
10	20	1.046	

RESULTS AND DISCUSSION

From the typical chromatogram of Nelfinavir Mesylate as shown in Figure 4, it was found that the retention time was 6.68 min. A mixture of Methanol and water in a ratio of 80:20 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.

CONCLUSION

The developed HPLC method for the estimation of Nelfinavir Mesylate was found to be simple and useful with high accuracy, precision, repeatability. Sample recoveries in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non Interference of formulation excipients in the estimation. In the selected solvent system methanol and water (80:20 v/v), drugs were stable for more than 48 hours, thus suggesting that samples need not be estimated immediately after collection. The developed method was found to be stability specific and were validated as per ICH guidelines (2008) and statistical method.

Acknowledgement
Authors are sincerely thankful to P.D.V.V.P.F'C College of Pharmacy Ahemadnagar for providing necessary facilities for work.

REFERENCES

[1] The Merck Index, XIII Ed., **2001**, Merck Research Laboratories, (Monograph No: 6471) pp 1154.

[2] S C Sweetman, Martindale-The Complete Drug Reference, 34th Ed., **2005** pp 650.
[3] E Y Wu, James M Wilkinson, Danel G Naret, Valerie L Daniels, Linda Jefferson Williams, Deborah A Khalil, and Bhasker V Shetty, *J.Chromatogr.B*, **1997**,**695**,pp 373.
[4] V Heeswijk , Hoetelmans R M W, R.Harns, Meenhorst P L , Mulder J W, Lange J M A, and Beijnen J H, *J.Chromatogr.B*, **1998**,**719**, pp159-168.
[5] A G Legrand , and Trihereau N, Bdiquet, *Chromatogr.B*, **2000**, **744**, pp227.
[6] V A Simon, Mamadou D and Thiam, *J.Chromatogr. A*, **2001**, **913**, pp 447-453.
[7] Paul Metz, Sue Kohlhepp J and Gilbert D N, *J.Chromatogr*.B, **2002**, **773, pp159**
[8] Michele L Turner, Kedria Reed-walker, Jennifer R King and Edward P Acosta, *J.Chromatogr*.B, **2003**,**784**, pp331-341.
[9] V A Frerichs and Robin Di Francesco, *J.Chromatogr.B*, **2003**, **787, pp393**-403.
[10] Katharina M Rentsch, *J.Chromatogr.B*, **2003**, **788**, pp 339-350.
[11] Rao Seshagiri, Mohan Reddy V M, Tadi R S Rao and Isukapatla N, *J. Anal. Chem*, **2004**, **59**(6), 552-556.
[12] R S Reo, Murali Mohan, Rama Subba Reddy T, Prasad Viplava U and Sastry C S P, *J. Institution of Chemists*; **2003**, **75**(2), pp 46.
[13] S V Reo, Murali Mohan, Reddy, Rama Subba T, Prasad Viplva U and Sastry C S P, *Asian J. Chem*, **2003**,**15** (2),pp 971.
[14] V Prakash . and Venkateswara Rao J, *E-J.Chem.*, **2006**, **3**, 78.
[15] K. Vanitha, E-*Journal of Chemistry* July **2007** Vol. 4, No. 3, pp. 302-306.
[16] D B Mohit. et al, *RJPT* Vol.04, Issue 10 Oct.**2011**