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# Development and validation of UV spectrophotometric method for estimation of nelfinavir in bulk and tablet dosage form

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**ABSTRACT**

*An simple, precise, accurate, reproducible, rapid, and economical UV Spectrophotometric method have been developed for estimation of nelfinavir in bulk and tablet dosage form by using methanol as solvent. Method devloped is Area Under Curve (AUC) method .For analysis of nelfinavir by AUC method the wavelength range selected was 250nm to 260nm because the linearity was obtained within these area with good reproducibility of results. Nelfinavir shows absorbance maxima (λmax) at 225nm when its appropriately dilluted solution was scanned in entire uv range of 400nm to 200nm. Nelfinavir obeys Beer's law in the concentration range of 5 to 40 ug/ml. Method is validated according to ICH guidelines and can be applied for routine analysis of drugs in tablet dosage form.*

**Keywords**: Nelfinavir, Anti-retroviral, Methanol, Area Under Curve (AUC) method, validation.

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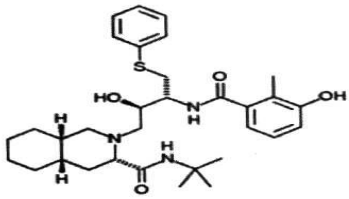
## INTRODUCTION

Nelfinavir is an Anti-retroviral drug belongs to the class of drugs known as protease inhibitors (PIs). It inhibits HIV-1 and HIV-2 proteases. Nelfinavir is chemically known as 2-[2-hydroxy-3-(3-hydroxy-2methyl-benzoyl)amino-4-phenylsulfanyl-butyl]-N-tert-butyl-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxamide.

Nelfinavir is a protease inhibitor: It inhibits HIV-1 and HIV-2 proteases. HIV protease is an enzyme (an aspartate protease) which splits viral protein molecules into smaller fragments, and it is vital to both the replication of the virus within the cell, and also to the release of mature viral particles from an infected cell. Nelfinavir is a competitive inhibitor (2 nM) which is designed to bind tightly and is not cleaved due to the presence of a hydroxyl group as opposed to a keto group in the middle amino acid residue mimic, which would be otherwise S-phenylcysteine. All protease inhibitors bind to the protease, the precise mode of binding determines how the molecule inhibits the protease. The way Nelfinavir binds the enzyme may be sufficiently unique to reduce cross-resistance between it and other PIs. Also, not all PIs inhibit both HIV-1 and HIV-2 proteases.



Nelfinavir

Nelfinavir was previously determined by Spectrophotometry [1-3], HPTLC [4], HPLC [5-7], and LCMS [8]. However no such simple, sensitive and précised spectrophotometric method is yet reported for this drug in any official literature. So in the present study, a specific, precise, accurate and validated spectrophotometric methods have been developed for the estimation of Nelfinavir in in bulk and tablet dosage form, using methanol as the solvent system.

## MATERIALS AND METHODS

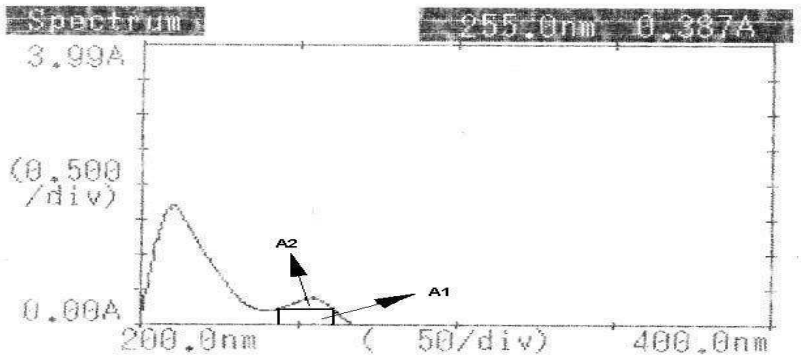
UV-Visible double beam spectrophotometer, Jasco model 2201 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 nm matched quartz cell was used. The commercially available tablets, Nelvir (Label claim: Nelfinavir- 250mg) was procured from local Market.

**Preparations of standard stock solution and calibration curve:**
Accurately about 10mg nelfinavir was weighed and transferred to 100ml volumetric flask. To it 50ml of methanol was added to dissolve the drug completely with vigorous shaking. Then the volume was made up with the same solvent up to the mark to give the standard stock solution of concentration 100µg /ml.

Working standard solutions of 10 ug/ml were scanned in the entire UV range of 400-200 nm to determine the λmax. The λmax of nelfinavir is 255nm. A series of volumetric flasks of 10ml capacity were arranged. To each of these flasks 0.5, 1, 2, 3, 4ml of the standard stock solution were added. The volume was made up with methanol. The area of these solutions was measured between 250nm to 260nm against reagent blank in spectrum mode. A calibration curve of area Vs concentration was plotted to obtain leniarity and regression equation.

**Area Under the Curve (AUC) method:**
The AUC (Area Under Curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 250nm and 260nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. By appropriate dilution of the standard stock solutions, working standard solutions of suitable concentrations were prepared accurately to determine the range for analysis (area under the curve). The standard solutions were then scanned in the spectrum mode of the instrument from 400nm to 200nm. The absorbance maxima of these solutions were found with a sharp peak at wavelength 255 nm (λmax of nelfinavir). The area under the curve between 250 nm to 260nm was selected (figure) for the calculation because the linearity was obtained within these area with good reproducibility of results. The area between 250 nm to 260 nm was measured for each solution.

The concentration range over which the drugs obeyed Beer-Lambert's law was chosen as the analytical concentration range. Here, the concentration range was found to be 5 to 40µg/ml for nelfinavir. The regression equation for nelfinavir was found to be $y = 0.12x + 0.1539$ and coifecient corelation($r^2$) was found to be $r^2 = 0.9981$. Using the regression equation, the unknown concentrations of the drug were determined in bulk and formulations.




**Wavelength range selected for AUC method of NFV**

**Analysis of tablet formulation:**

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 10 mg of nelfinavir was transferred to 100 ml volumetric flask and dissolved in methanol and final volume was made up with the same. The sample solution was then filtered through Whatman filter paper no.41. This is stock solution of 100 ug/ml. From the above stock solution 0.5, 1, 2, 3, 4ml of solution was transferred in 10 ml volumetric flask and was diluted with methanol upto 10 ml. This gives solution of 5 to 40ug/ml concentration of nelfinavir. These solutions were scanned under entire uv region (400nm to 200nm) and area of it between the wavelength range 250nm to 260nm was calculated to get the concentration of drugs. Analysis procedure was repeated six times with tablet formulation. The results of tablet analysis are reported in table.

**Validation of developed method**

**Linearity:**

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed method. For the developed method the Beer-Lambert's concentration range was found to be 5 to 40ug/ml for nelfinavir. The linearity data of the method are presented in table 1 and 2.

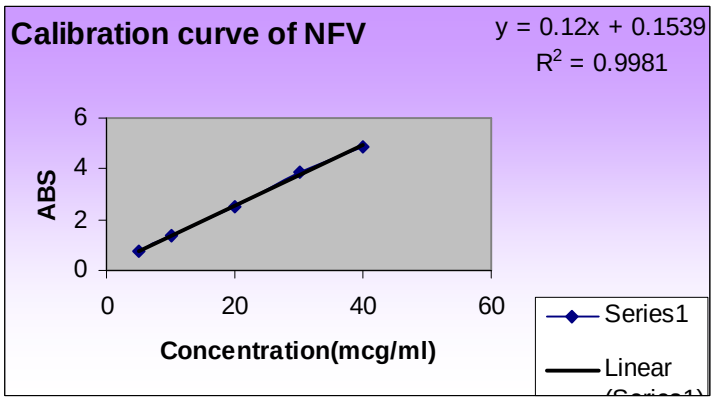



**Table 1: Standard calibration table for Nelfinavir in AUC method**

| Sr. No. | Concentration (µg/ml) | AUC |
|---|---|---|
| 1 | 5 | 0.7419 |
| 2 | 10 | 1.3569 |
| 3 | 20 | 2.5099 |
| 4 | 30 | 3.8807 |
| 5 | 40 | 4.8852 |

**Table 2: Statistical validation of calibration curve of Nelfinavir by AUC method**

| Title | Mean | S.D. | S.E.M. |
|---|---|---|---|
| Slope | 0.1278 | 0.004114 | 0.001679 |
| Y-intercept | 0.05835 | 0.04406 | 0.01799 |
| $R^2$ Value | 0.9977 | 0.002857 | 0.001166 |

**Precision**

**Repeatability:**

To check the degree of repeatability of these methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablet formulation. The standard deviation, coefficient of variation and standard error was calculated. The results of statistical evaluation are given in table 4.

**Accuracy:**

To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are shown in table 5 and 6.

To perform recovery studies at 80% of the test concentration, a pre analyzed tablet sample containing 10 mg of nelfinavir was weighed. To it 8 mg of standard nelfinavir was added, the mixture was mixed thoroughly. From this pool, sample powder containing quantity equivalent to 10 mg of nelfinavir was weighed and transferred to a 100 ml volumetric flask. To it 50ml of methanol was added and the content was kept for ultrasonication to shake. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper . The sample mixture was then analyzed as per the procedure given for tablets.

Similarly to perform recovery studies at 100% and 120% of the test concentration , a preanalyzed tablet sample containing 10mg of nelfinavir was weighed. To it 10 mg of standard nelfinavir and 12mg of standard nelfinavir respectively was added separately. The powders were mixed properly. From this pool, sample powder containing quantity equivalent to 10 mg of nelfinavir was weighed separately for 100% recovery and 120% recovery respectively. The powder then transferred to 100 ml volumetric flasks separately. The sample dilution and analysis was performed as per the procedure given as above The recovery study was performed three times at each level for the tablet formulations. The results of the recovery studies along with its statistical validation are given in the Table 5 and 6.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):**

The LOD and LOQ of nelfinavir by proposed method was determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are quoted in results and discussion.

## RESULTS AND DISCUSSION

Linearity range of nelfinavir is 5 to 40ug/ml at calculated area between wavelength range from 250nm to 260nm. The coefficient of correlation for nelfinavir at calculated area is 0.9981. Nelfinavir shows good regression values at their respective calculated area and the results of recovery study revealed that any small change in drug concentration in the solution could be accurately determined by the proposed method.

Percentage estimation nelfinavir from tablet dosage form by this method is 99.80 with standard deviation ≤2. (Table 3).

Precision is determined by studying the repeatability. Repeatability result indicates the precision under the same operating condition over a short interval of time and interassay precision. The standard deviation, coefficient of variance and standard error is calculated for nelfinavir. The result is quoted in Table 4.

The validity and reliability of proposed methods is assessed by recovery studies. Sample recovery for this method is in good agreement with the respective label claim, which suggest non interference of formulation additives in estimation. Table 5 and 6

The LOD value is 0.2010ug/ml while LOQ value is 0.4501ug/ml in proposed method for nelfinavir. Low values of LOD and LOQ indicates good sensitivity of proposed methods.

**Table 3 : Analysis of tablet formulation**

| Sr. No. | Tablet Sample Name | Amount Present (mg /tab) | Amount Taken (mg) | Amount found (mg / tab) | Percentage of label claim (%) |
|---|---|---|---|---|---|
| 1 | Nelfinavir | 250 | 10 | 9.972 | 99.72 |
| 2 | | 250 | 10 | 9.995 | 99.95 |
| 3 | | 250 | 10 | 10.012 | 100.12 |
| 4 | | 250 | 10 | 9.986 | 99.86 |
| 5 | | 250 | 10 | 9.938 | 99.38 |
| 6 | | 250 | 10 | 9.982 | 99.82 |

**Table 4 : Statistical evaluation by AUC method**

| Sr. No. | Tablet Sample Name | % Mean * | S.D.* | C.O.V.* | S.E.* |
|---|---|---|---|---|---|
| 1 | Nelfinavir | 99.80 | 0.2495 | 0.25 | 0.1019 |

**Average of six readings*

The % mean, standard deviation (S.D.), coefficient of variation (C.O.V.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The C.O.V. is also less than 2% as required by USP and ICH guidelines.

**Table 5 : Recovery Studies**

| Tablet Sample Name | Level of Recovery (%) | Amount present (mg/tab) | Amount Taken (mg) | Amount of Std Added (mg) | Total Amount recovered (mg) | % Recovery |
|---|---|---|---|---|---|---|
| **Nelvir** | 80 | 250 | 10 | 08 | 17.99 | 99.93 |
| | | 250 | 10 | 08 | 18.007 | 100.04 |
| | | 250 | 10 | 08 | 17.98 | 99.92 |
| | 100 | 250 | 10 | 10 | 20.01 | 100.05 |
| | | 250 | 10 | 10 | 19.93 | 99.65 |
| | | 250 | 10 | 10 | 19.98 | 99.89 |
| | 120 | 250 | 10 | 12 | 22.08 | 100.39 |
| | | 250 | 10 | 12 | 21.98 | 99.90 |
| | | 250 | 10 | 12 | 21.99 | 99.97 |

**Table 6 : Statistical validation of recovery studies**

| Sr. No. | Tablet Sample Name | Level of Recovery (%) | (%) Mean* | S.D.* | C.O.V.* | S.E.* |
|---|---|---|---|---|---|---|
| 1 | **Nelvir** | 80 | 99.96 | 0.0666 | 0.6660 | 0.03844 |
| 2 | | 100 | 99.86 | 0.2013 | 0.2015 | 0.1162 |
| 3 | | 120 | 100.09 | 0.2650 | 0.2647 | 0.1530 |

*Where *n=3 at each level of recovery.*

The result of the recovery studies indicates high degree of accuracy of the proposed method.

## CONCLUSION

The developed method is sensitive, unique, precise, user friendly, fast and reproducible for estimation of nelfinavir in bulk mix and Pharmaceutical dosages forms. The method is validated as per the ICH Guidelines. It is concluded that this method can be used by the industries and academic institutions for their drug estimation, which is rapid as well as novel.

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