Nangare Ajit et al. / Journal of Pharmacy Research 2014,8(1),68-72

Research Article
ISSN: 0974-6943

Available online through
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Novel UV spectrophotometric method for estimation of Nelfinavir by derivative spectroscopy

Nangare Ajit[1]*, Hapse Sandip[2]
[1]Department of pharmaceutical chemistry, Padmashree Dr.Vittalrao Vikhe Patil College of Pharmacy, Ahmednagar, Maharashtra. 414111,India.
[2]Department Of Quality Assurance Technique,Padmashree Dr.Vittalrao Vikhe Patil College of Pharmacy, Ahmednagar, Maharashtra. 414111,India..

Received on:21-11-2013; Revised on: 08-12-2013; Accepted on:11-12-2013

ABSTRACT

Two Simple, precise and economical UV methods have been developed and validated for the quantitative estimation of Nelfinavir in bulk and pharmaceutical dosage forms. Nelfinavir showed sharp peak at lmax 255 nm (Method A, zero order), showed sharp peak at 248 nm (Method B, first order). Methanol was used as solvent for all methods. Beer's-Lamberts law was found to be obeyed in the concentration range of 5-40 µg/ml. The developed methods were statically validated according to international conference on harmonization guidelines and were found to be accurate and reproducible. Results of the analysis were validated statistically and by recovery studies

KEYWORDS: Nelfinavir, Derivative spectroscopy

INTRODCTION

Direct UV–visible spectrophotometric method is not suitable for simultaneous determination of Nelfinavir due to their spectral overlapping in the region of 200- 400 nm. Application of derivative technique of spectrophotometry offers a powerful tool for quantitative analysis Nelfinavir is an Anti-retroviral drug and is Chemically (3S,4aS,8aS)-N-tert-butyl-2-[(2R,3R)-2- hydroxy-3- [(3-hydroxy -2-methylphenyl) formamido]- 4-(phenylsulfanyl)butyl] decahydro isoquinoline-3-carboxamide. Nelfinavir belongs to the class of drugs known as protease inhibitors (PIs). And inhibits HIV-1 and HIV-2 proteases. Nelfinavir was previously determined by Spectrophotometry [1, 2, 3] HPTLC 4, HPLC [5, 6, 7, 8] and LCMS [9]. However no such simple, sensitive and précised spectrophotometric method is yet reported for this drug in any official literature. So in the present study, specific, precise, accurate and validated spectrophotometric methods have been developed for the estimation of Nelfinavir in bulk and tablet dosage form, using methanol as the solvent system.

INSTRUMENTATION

A double-beam Shimadzu UV- 1601; UV Visible spectrophotometer, spectral bandwidth of 2nm, wavelength accuracy ±0.5nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution.

MATERIAL AND METHOD

Materials

Standard sample of Nelfinavir were obtained and Nelfinavir tablets (containing 250mg of Nelfinavir), were purchased from local market.

***Corresponding author.**
Prof. Nangare Ajit
Department of Pharmaceutical Chemistry, Padmashree Dr.Vittalrao Vikhe Patil College of Pharmacy, Ahmednagar, Maharashtra. 414111,India.

PROCEDURE

1. Zero order spectrum method

1) A. Preparations of drug stock solution:

Accurately about 10mg NFV 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 drug stock solution of concentration. 100 µg /ml.

B. Preparations of standard drug dilutions:

From the stock solution of NFV appropriate volumes of different concentrations were pipette out and transferred to 10 ml volumetric flask. The volume was made up to the mark with methanol to give the samples of desired concentrations as shown in **figure 1**.

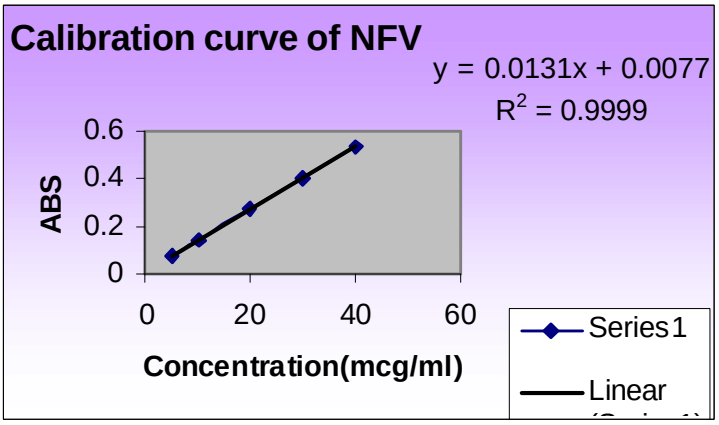

Figure 1: Calibration curve of NFV

2. Selection of analytical wavelengths:

The dilutions of **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 (λ_{MAX} of NFV in the solvent selected) as shown in **figure 2**.

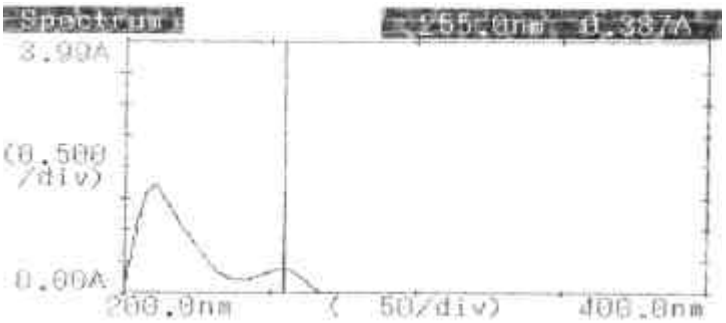

Figure 2: Zero order spectrum of NFV

3) Preparation of working curve:

A series of volumetric flasks of 10ml capacity were arranged. To each of these flasks 0.5,1,2,3,4, ml of the drug stock solution was added. The volume was made up with methanol. The absorbance of the peak in zero order spectrums of these solutions were measured at 255 nm against reagent blank as shown. A linear graph of absorbance of the peak Vs concentration was obtained passing through the origin, which shows that there was an increase in absorbance with increasing concentration of the drug. 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 40 µg / ml for NFV. The standard calibration table and curve for NFV are given in the **Table 1**.

Table 1: Standard calibration table for NFV

Sr. No	Concentration (µg/ml)	Absorbance
1	5	0.073
2	10	0.140
3	20	0.270
4	30	0.399
5	40	0.534

4) Estimation of NFV in pharmaceutical dosage form:

For estimation of NFV in tablet formulations, tablets of each brand were weighed and triturated to fine powder. Tablet powder equivalent to 10 mg of NFV was weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol. It was kept for ultrasonification for 45 min. Finally the volume was made up to the mark with quantity sufficient methanol; this was then filtered through Whatmann filter paper to get tablet stock solution of concentration of 100 µg /ml. Then suitable aliquots of 100 µg/ml tablet stock solution were diluted with methanol to get the final dilutions .These were Analyzed at 255 nm against reagent blank in quantitation mode. The procedure was repeated six times for each tablet formulations. The results of the analysis of tablet formulations as shown in **Table 2**.

Table 2: Analysis of tablet formulations

Sr. No.	Tablet Sample Name	Amount Present (mg /tab)	Amount found (mg / tab)	Percentage of label claim
1		250mg	252.37	100.94%
2		250mg	252.00	100.80%
3	Nelfinavir	250mg	247.54	99.02%
4		250mg	252.53	101.01%
5		250mg	254.98	101.90%
6		250mg	253.18	101.27%

7) Statistical validation:

To check the degree of precision of the method, suitable statistical evaluation was carried out. Six samples of the each tablet formulation were analyzed as per the procedure given above. The standard deviation (S.D.), coefficient of variation (C.O.V.) and standard error (S.E.) were calculated. The results of the statistical evaluation are given in **Table 3**.

Table 3: Statistical evaluation by zero order spectrum method

Sr. No.	Tablet Sample Name	% Mean *	S.D.*	C.O.V.*	S.E.*
1	Nelfinavir	100.84	0.9898	0.9815	0.4041

8) Recovery studies:

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. To perform recovery studies at 80% of the test concentration, a pre analyzed tablet sample containing 10 mg of NFV was weighed. To it 8 mg of standard NFV was added, the mixture was mixed thoroughly. From this pool, sample powder containing quantity equivalent to 10 mg of NFV 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 (4). Similarly to perform recovery studies at 100% and 120% of the test concentration, a preanalyzed tablet sample containing 10mg of NFV was weighed. To it 10 mg of standard NFV and 12mg of standard NFV respectively was added separately. The powders were mixed properly. From this pool, sample powder containing quantity equivalent to 10 mg of NFV 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**.

Table 4: 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
	80	250	10	08	18.17	100.94
		250	10	08	18.14	100.77
		250	10	08	18.32	101.77
	100	250	10	10	19.98	99.90
Nelfinavir		250	10	10	19.99	99.95
		250	10	10	20.02	100.10
	120	250	10	12	22.06	100.27
		250	10	12	22.33	101.50
		250	10	12	22.34	101.54

Table 5: Statistical validation of recovery studies

Sr. No.	Tablet Sample Name	Level of Recovery (%)	(%)Mean*	S.D.*	C.O.V.*	S.E.*
1	Nelfinavir	80	101.19	0.5437	0.5373	0.3139
2		100	100.27	0.6327	0.6309	0.3653
3		120	101.13	0.7160	0.7079	0.4134

Where *n=3 at each level of recovery.

II First Order Derivative Spectroscopy-

1) A. Preparations of drug stock solution:
Standard stock solutions were prepared as discussed in the previous method.

B. Preparations of final dilutions:
Final dilutions from the stock solution of NFV were prepared as discussed in the previous method.

2) Selection of analytical wavelength:
By appropriate dilution of the standard stock solutions working standard solutions of suitable concentrations were prepared accurately. The standard solutions were then scanned in the spectrum mode of the instrument from 400nm to 200nm. The first order derivative spectrum was obtained with wavelength difference (n=1). To obtain good results, the wavelength selected should be such that at this wavelength, the absorptivity should be as large as possible. The absorbance maxima of these solutions were found with a sharp peak at 248 nm (λ_{MAX} of NFV in first order derivative spectrum) and zero crossing at 255 nm (λ_{MAX} of NFV in zero order spectrums). The absorbance of the peak at 248nm was measure for each solution as shown in **Figure 3.**

3) Preparation of working curve: A series of volumetric flasks of 10ml capacity were arranged. To each of these 0.5, 1, 2, 3, 4 ml of the drug stock solution were added. The volume was made up with methanol. The absorbance of the peak in first order derivative spectrum with n=1 of these solutions were measured at 248 nm against reagent blank. A linear graph of absorbance of the peak Vs concentration was obtained passing through the origin, which shows that there was an increase in absorbance with increasing concentration of the drug. The concentration range over which the drugs obeyed Beer-Lambert's law was chosen as the analytical concentration range (5 to 40 µg/ml) for NFV. The standard calibration table and curve for NFV in first order derivative spectrum with n=1 is given in **Figure 4**.

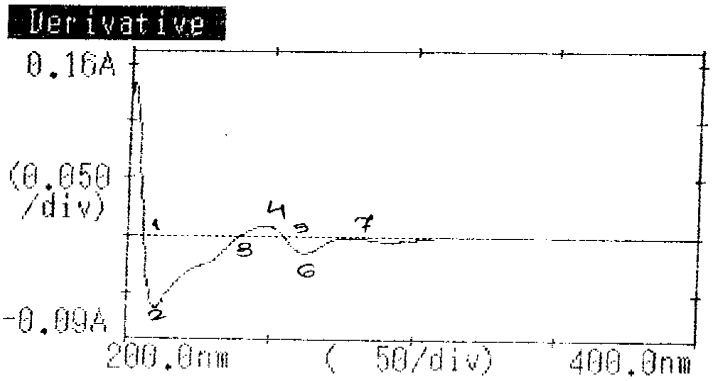

Figure 3: First order derivative spectrum of NFV with n=1

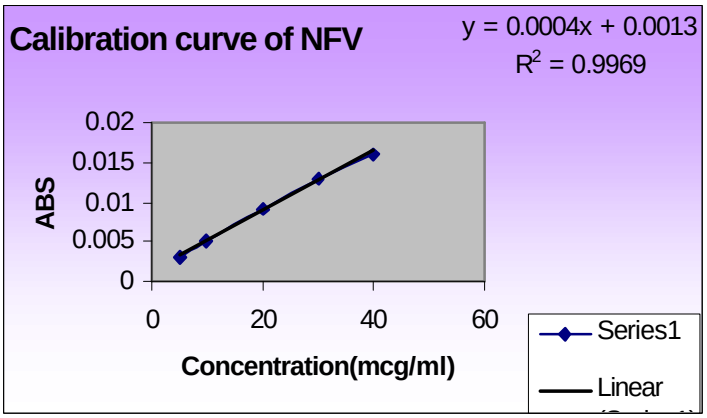

Figure 3: Calibration curve of NFV in first order derivative method

5) Estimation of NFV in pharmaceutical dosage form:

For estimation of NFV in table formulations in first order derivative spectrum method, the same procedure was followed to make the stock solution of various dilutions for each brand of tablet formulations. Analysis of NFV in tablet formulation was done in above concentration at 248nm using quantitation mode in first order derivative spectrum with n=1 against reagent blank. The procedure was repeated six times for each tablet formulations. The results of the analysis of both the tablets formulations are give in **Table 6**.

Table 6: Analysis of tablet formulation.

Sr. No.	Tablet Sample Name	Amount Present (mg /tab)	Amount found (mg / tab)	Percentage of label claim (%)
1	Nelfinavir	250	252.68	101.07
2		250	254.22	101.69
3		250	253.96	101.58
4		250	251.27	100.51
5		250	254.63	101.85
6		250	251.60	100.64

6) Statistical validation:

To check the degree of precision of the method, suitable statistical evaluation was carried out. Six samples of the each table formulation were analyzed as per the procedure given under Tablets. The standard deviation (S.D.) coefficient of variation (C.O.V.) and standard error (S.E.) were calculated. The results of the statistical evaluation are given in **Table 7**.

Table 7: Statistical evaluation by first order derivative

Sr. No.	Tablet Sample Name	% Mean *	S.D.*	C.O.V.*	S.E.*
1	Nelfinavir	101.22	0.5675	0.5606	0.2317

7) Recovery studies:

The recovery studies were performed in a way similar to the previous method. The results of the recovery studies along with its statistical validation are given in **Table 8, 9**.

Table 8: 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
Nelfinavir	80	250	10	08	18.05	100.27
		250	10	08	18.31	101.72
		250	10	08	18.01	100.05
	100	250	10	10	20.05	100.25
		250	10	10	20.13	100.65
		250	10	10	19.98	99.90
	120	250	10	12	22.37	101.68
		250	10	12	22.33	101.50
		250	10	12	22.23	101.04

Table 9: Statistical validation of recovery studies

Sr. No.	Tablet Sample Name	Level of Recovery (%)	(%) Mean*	S.D.*	C.O.V.*	S.E.*
1	Nelfinavir	80	100.69	0.9349	0.9284	0.5398
2		100	100.27	0.3868	0.3857	0.2233
3		120	101.43	0.3164	0.3119	0.1827

Where *n=3 at each level of recovery.

RESULT AND DISCUSSION

From the proposed method, it was found that Nelfinavir obeys linearity within the concentration range 1-40µg/ml. Percentage label claim for Nelfinavir by both the methods was found in the range of 100.94% to 100.69%. For both method. Coefficient of variation (CV) were calculated, which was found to be less than 2% indicating the method has good reproducibility. Accuracy of proposed methods was ascertained by recovery studies and results are expressed as %recovery. Percent recovery for Nelfinavir by both methods was found in range of 101.77% to 101.72%, values of standard deviation and coefficient of variation were in range of 0.9898 to 0.5675 and 0.0.9815 to 0.5606 respectively indicating the accuracy of proposed method.

ACKNOWLEDGEMENT:

The author is thankful to Padmashree Dr.Vithalrao Vikhe Patil College of Pharmacy, Ahmednagar (MH) for providing necessary facilities to carry out research work.

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Source of support: Nil, Conflict of interest: None Declared