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Spectrophotometric Method for estimation of Nelfinavir mesylate

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Abstract: Three Simple, precise and economical UV methods have been developed and validated for the quantitative estimation of Nelfinavir mesylate in bulk and pharmaceutical dosage forms. Nelfinavir mesylate showed sharp peak at λ_{max} 254 nm (Method A, zero order), showed sharp peak at 247 nm (Method B, first order) and in the second order spectra (Method C) at 233 nm . Methanol was used as solvent for all the three methods. Beer's-Lamberts law was found to be obeyed in the concentration range of 10-50 µg/ml. The developed methods were stastically validated according to International Conference on Harmonization Guidelines and was found to be accurate and reproducible. Results of the analysis were validated statistically and by recovery studies.
Keywords: Nelfinavir mesylate, Methanol, Derivative Spectroscopy, absorption maximum, Antiviral.

Introduction

Nelfinavir mesylate 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 mesylate 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, a specific, precise, accurate and validated spectrophotometric methods have been developed for the estimation of Nelfinavir mesylate in in bulk and tablet dosage form, using methanol as the solvent system.

Materials and Methods:

Instruments and reagents

An analytically pure sample of Nelfinavir mesylate was procured as gift sample from Matrix laboratories (Hyderabad, India). Analytical grade methanol was used as solvent for dilution. Shimadzu UV-1800 UV/VIS spectrophotometer was used with 1 cm matched quartz cell. The tablet formulation [NELVIR, Hetero Drugs Limited, Hyderabad, India] was procured from a local pharmacy with labelled amount 625 mg per tablet.

Preparation of working standard

Standard Nelfinavir mesylate 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to

give a solution containing 1000 µg / ml. From this stock solution, pipetted out 10 ml and placed into 100 ml volumetric flask. The volume was made up to mark with methanol to give a solution containing 100 µg / ml, which was then used as stock solution for the studies.

Analysis of marketed formulation

Twenty tablets were weighed and finely powdered. The powder equivalent to 100 mg of Nelfinavir mesylate was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 25 ml of the methanol and sonicated for 5 min. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg / ml. The above solution was centrifuged at 2000 rpm for 10 minutes and carefully filtered through Whatmann filter paper (No. 41). From this solution, 10ml was taken and diluted to 100 ml methanol to give a solution of 100 µg / ml and used for the analysis of Nelfinavir mesylate.

Recovery studies and validation of the method according to ICH guidelines

Precision of the newly developed methods were studied by carrying out intraday, interday analysis and expressed as per cent coefficient of variance [10]. Specificity of the method was checked by adding few excipients within the range as specified in standard literature which are usually added in the marketed preparations such as diluents, lubricant *etc*. to the preanalysed sample. The absorbance of the solution so obtained after addition of excipients was than measured, compared with that of the absobance of preanalysed solution and specificity was expressed in terms of percent interference, which was found to be less than 2 %. Limit of detection (LOD) and limit of Quantification (LOQ) were studied based on standard deviation of the response and slope curve. Recovery studies were carried out by addition of standard drug to preanalyse samples of marketed formulation, taking in to consideration the % purity of the added bulk drug.

Table 1: Optical characteristics and Other Parameters

Parameters	Results		
	Method A	**Method B**	**Method C**
Absorption Maxima (nm)	254	247	233
Beer's-Lambert's range (µg/ml)	10-50	10-50	10-50
Regression equation (y)*			
Slope (b)	0.0139	0.0014	0.0005
Intercept (a)	0.0046	0.0004	1E-04
Correlation coefficient	0.9995	0.9996	0.9991
Sandell's sensitivity (mcg / cm^2-0.001 absorbance units)	0.071429	0.5760	1.875
Intraday precision (% RSD)	1.24	1.47	2.83
Interday precision (% RSD)	0.96	1.21	3.51
Accuracy	99.34±0.21	98.83±0.63	99.60±0.53
Limit of detection (µg / ml)	0.19	0.75	2.28
Limit of quantification (µg / ml)	0.59	2.29	6.92

*y = bc +a: when c is the concentration in mg/ml and y is absorbance unit.

Table 2: Analysis of tablet formulation

Method	Tablet	Label claimed (mg)	Amount found (mg)	%Recovery ± SD**
A	Tablet I	625	99.50	100.92 ± 0.17
	Tablet II	625	99.84	101.36 ± 0.09
B	Tablet I	625	100.34	100.47 ± 0.19
	Tablet II	625	99.76	99.91 ± 0.46
C	Tablet I	625	99.42	99.94 ± 0.23
	Tablet II	625	99.61	99.87 ± 0.31

**Average of six determinations.

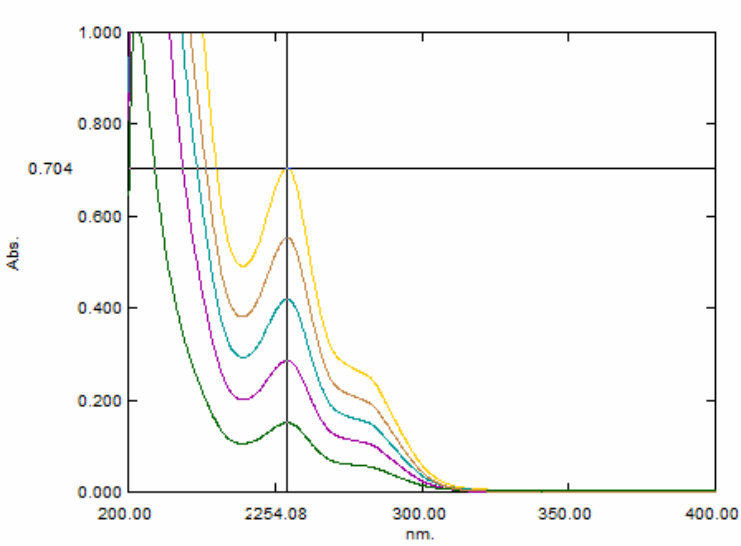

Fig: 1 - Zero order spectra of Nelfinavir mesylate at 254 nm

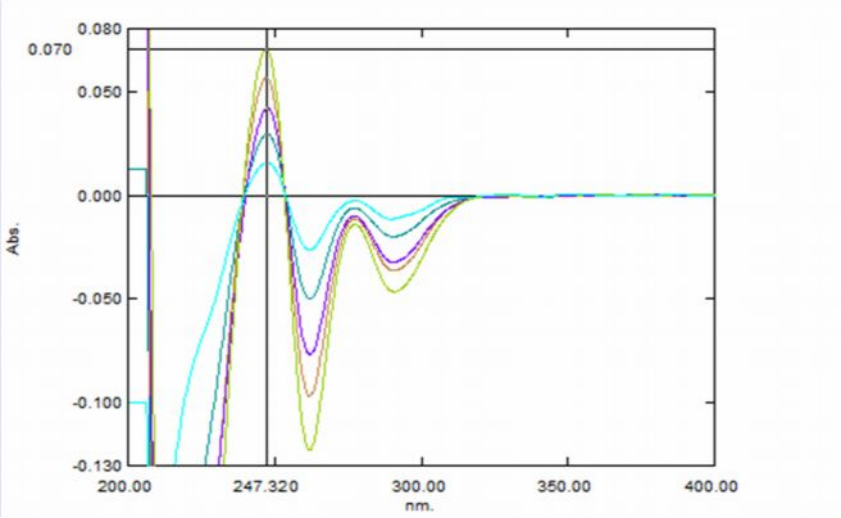

Fig: 2- First order spectra of Nelfinavir mesylate at 247 nm

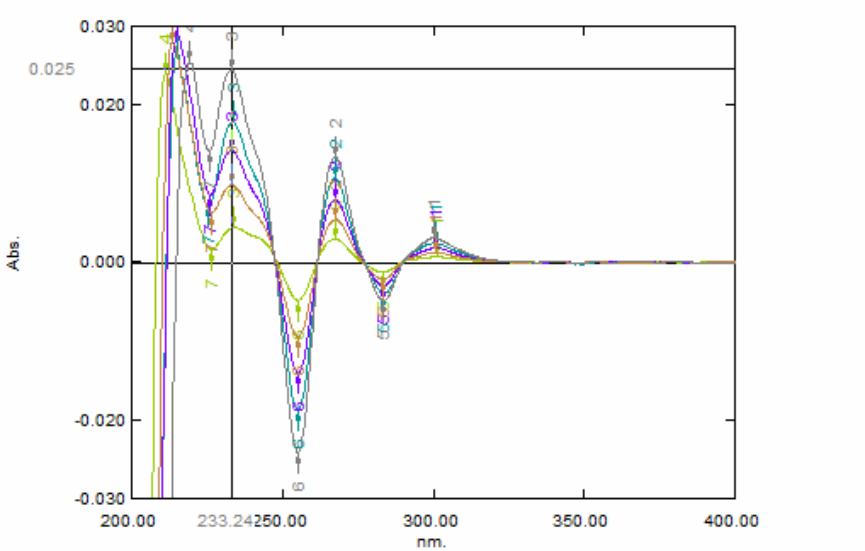

Fig: 3-Second order spectra of Nelfinavir mesylate at 233 nm

Results and Discussion

Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivatized from Zero, first and Second order. The first and second order derivative spectrum was selected for the analysis of the drug. The Nelfinavir mesylate shows absorption maxima a 254 nm, 247 nm and 233 nm for Zero (Method A), First (Method B) and Second (Method C) order derivative spectroscopy are shown in Fig:1, 2 and 3. The linear regression equation for Nelfinavir mesylate standard curve was calculated for all the three methods. The value of regression coefficient depicts the linearity of the data range and for the given data it shows that Beer-Lambert's Law follows a linear relationship for Nelfinavir mesylate in the range of 10-50 µg/ml.

For precision, repeatability, intraday/interday, three replicate experiments were carried out and their % RSD readings were calculated at the selected λ_{max} . All the Characteristic parameters were shown in Table 1 and the results of marketed formulation is shown in Table 2.

4. Conclusion

The proposed methods are validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and relatively inexpensive. The developed methods can be easily applied for the routine Quality Control analysis of Nelfinavir mesylate in bulk and pharmaceutical preparations.

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