Research Article **CODEX: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

DEVELOPMENT AND VALIDATION OF DIFFERENTIAL SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF NELFINAVIR MESYLATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid and sensitive difference spectrophotometric method was used for the determination of Nelfinavir mesylate in pharmaceutical dosage forms. The method is based on the induced spectral changes upon changing the pH of the medium that differ in their UV spectra. Difference spectrum, obtained by keeping Nelfinavir mesylate in 0.1N Hcl in reference cell and Nelfinavir mesylate in 0.1N NaOH in sample cell, showed two characteristic peaks at 214nm and 236nm with positive and negative absorbance respectively. Difference of absorbance between these two maxima was calculated to find out the amplitude, which was plotted against concentration. The calibration curve is linear over the concentration range of 10-60 µg/ml (r^2= 0.996). The method was successfully applied to the commercial pharmaceutical drug without interference from common ingredient accompanying the drug. The result statistically compared with those obtained by the reference method. The proposed methods were successfully applied to the assay of Nelfinavir mesylate in pure and tablet dosage form. No interference was found from tablet excipients at the selected wavelengths and assay conditions. The data were compared with those obtained from the spectrophotometric method given in the literature and no difference was found statistically.

KEY WORDS

Difference Spectroscopy, Nelfinavir mesylate and Tablet.

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INTRODUCTION

Nelfinavir mesylate (Figure No.1) 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 as given in Figure No.1. 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[1-3], HPTLC[4], HPLC[5-8] 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 mesylate in bulk and tablet dosage form using methanol as the solvent system.

Literature survey reveals large number of chromatographic methods for the estimation of Nelfinavir mesylate in various biological fluids[10-12] and in combination with the other antiviral[13]. However very few spectrophotometric methods are reported till date for this drug. So for the routine quality control analysis of various pharmaceutical formulations, there is a need for fast, low cost and selective method. Therefore in the present study, a spectroscopic method has been developed for the estimation of Nelfinavir mesylate in the bulk and tablet dosage form using the combination of methanol and water as solvent system in the ratio 1:1. The main purpose of the present study was to establish a relatively simple, single-step, sensitive, validated and inexpensive spectrophotometric method for the determination of Nelfinavir mesylate in pure form and in pharmaceutical dosage form, since most of the previous methods have been found to be relatively complicated and expensive, such as HPLC and CE.

EXPERIMENTAL CONDITION

Materials and Methods

A Lab India UV-Vis Spectrophotometer 3000^{+} with 1.0 cm matched quartz cells was used for all spectral measurements. Nelfinavir mesylate bulk drug was obtained from Cipla Pvt. Ltd, Hyderabad, India. Viracept tablets (250mg) were obtained from the market, manufactured by Cipla Ltd., Haridwar, India. Sodium hydroxide and Hydrochloric Acid (0.1N Solution), Water was double distilled.

PROCEDURE

Calibration

Stock Nelfinavir Mesylate solution was prepared by dissolving 100 mg of working standard in 100ml of Distilled Water. Working standard solutions with concentration ranging from 10-60 µg/ml in methanol were prepared by transferring appropriate volume of stock solution to 25 ml volumetric flask in duplicate. The volume was then adjusted with 0.1N Hcl and 0.1N NaOH to give a series of equimolar solutions of Nelfinavir Mesylate in different pH medium. Difference spectra were obtained by keeping acidic form (0.1N Hcl) in reference cell and basic form (in 0.1N NaOH) in sample cell. Difference of absorbance between 214 nm and 236 nm was calculated to find out the amplitude (Table No.1).

Calibration plot

A plot of difference absorbance Vs Nelfinavir Mesylate concentration was seen to be linear over the concentration range 10-60µg/ml (r^2 =0.996) with a slope of 0.002 and intercept of 0.046 (Figure No.2).

Procedure for the assay of Nelfinavir mesylate in tablet

The average mass of 20 tablets was determined and was ground in a mortar. An amount of powder (accurately weighed) equivalent to 250 mg Nelfinavir mesylate was transferred in 100ml volumetric flask and made up to the mark with NaOH and Hcl. The content of the flask was sonicated for 10min and then the solution was filtered through Whatmann no-1 filter paper. The flask gradually was shaken and then solution was made up to the mark. The volume was then adjusted with 0.1N Hcl and 0.1 N NaOH. The Absorbance Difference (ΔA) between the acidic solution and basic solution was measured at 214nm and 236 nm by placing acidic solution as reference and basic solution as sample. The content of the tablet is calculated from the calibration curve or using the corresponding regression equation in (Table No.2).

Interference studies

The effect of foreign substances, inactive excipient material that commonly accompanying the drug in pharmaceutical formulation such as tablets (starch,

mannitol, cellulose, PVP, magnesium stearate, absorption spectra of Nelfinavir mesylate in standard solution and in solution at some extract (for example: Viracept 250 mg). The obtained absorption spectra are identical.

RESULT AND DISCUSSION

This work describes a simple pH induced difference spectrophotometric method for the determination Nelfinavir mesylate in tablets (in the presence of excipients). The absorbance spectra of equimolar solutions of Nelfinavir mesylate in 0.1N Hcl (pH 1) and 0.1N NaOH (pH 11), are shown in Figure No.3.

Figure No.4 shows the difference absorption spectrum of Nelfinavir mesylate solution. It is generated by measure the absorbance of equimolar Nelfinavir mesylate solution at pH 11 (in 0.1N NaOH) in sample cell against the Nelfinavir mesylate at pH 1 (in 0.1N Hcl) form in reference cell.

titanium dioxide) was studied by comparision of the The proposed method was validated with respect to linearity, precision and accuracy according to ICH guidelines[13]. The accuracy of the proposed method was evaluated by recovery studies (standard addition method) at three different levels. The results of the recovery studies are given in Table No.2. For precision of method, six standard solutions were evaluated at same day as well as at different days.

Summary of all the validation parameters are given in Table No.2.

Analysis of commercial tablets

Difference spectrophotometric method was applied to brand of Nelfinavir mesylate tablet well known in the market. The result of analysis is reported in Table No.2. The reproducibility of the method was checked by five replicate determinations and then the Relative standard deviation (RSD) is calculated.

Table No.1: Concentration and Absorbance of Nelfinavir Mesylate in Acidic and alkaline medium (Amplitude)

S.No	Concentration (mcg/ml)	Absorbance		Amplitude
		214nm	236nm	(Difference)
1	10	0.072	-0.043	0.115
2	20	0.092	-0.138	0.230
3	30	0.125	-0.174	0.299
4	40	0.146	-0.232	0.378
5	50	0.172	-0.305	0.477
6	60	0.195	-0.365	0.560

Table No.2: Regression analysis and Validation Parameter

S.No	Parameter	UV method
1	Beer's law limits (mcg / ml)	10-60
2	Regression equation (Y*)	Y = 0.002X - 0.046
3	Slope (b)	0.002
4	Intercept (a)	0.046
5	Correlation coefficient(r^2)	0.996
6	% RSD**	< 2%
7	Intraday %	1.0-1.7
8	Interday %	1.0-1.8
9	% Recovery(Tablet)	100.45%

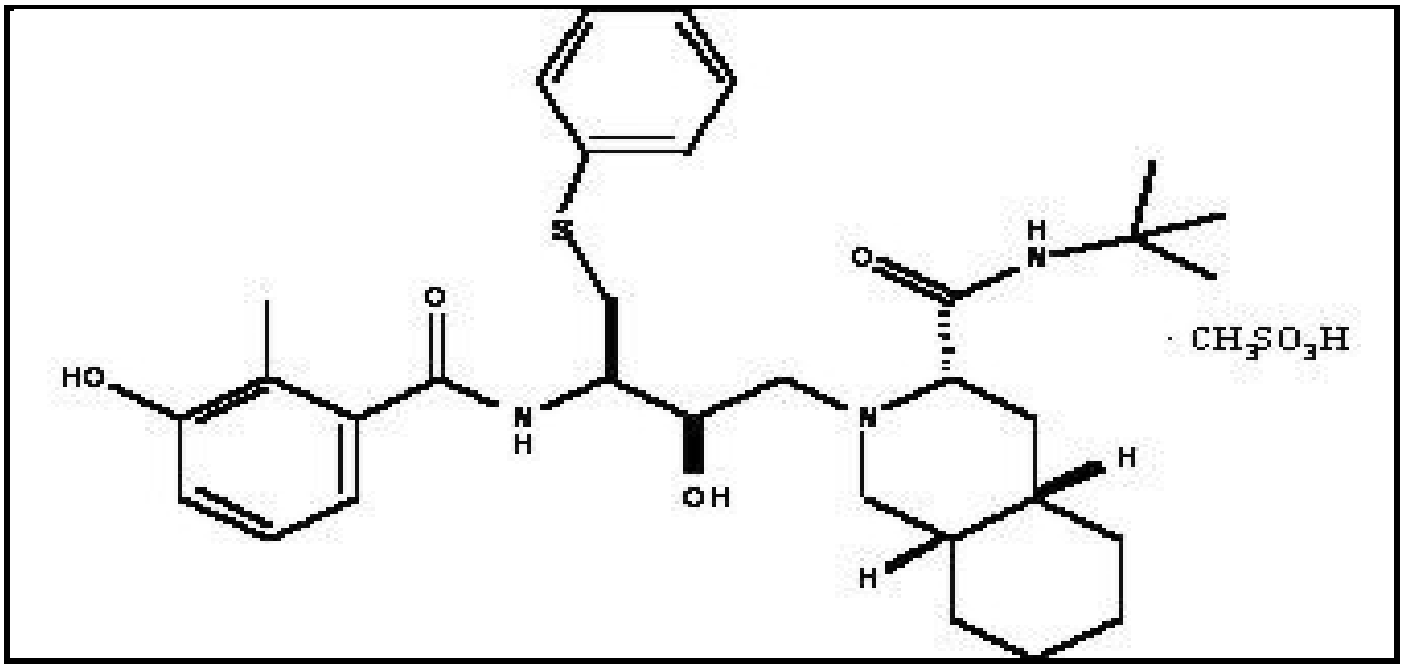

Figure No.1: Structure of Nelfinavir mesylate

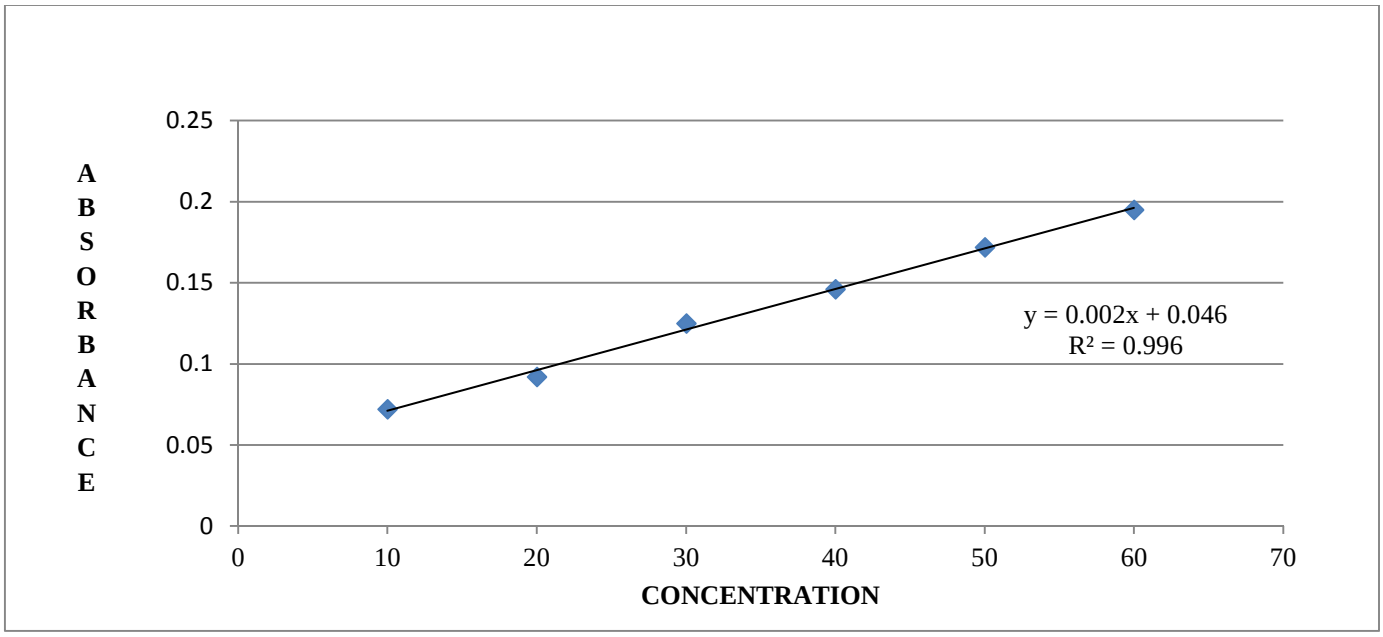

Figure No.2: The Difference Absorption Calibration Curve of Nelfinavir Mesylate in 0.1N NaOH and 0.1N Hcl

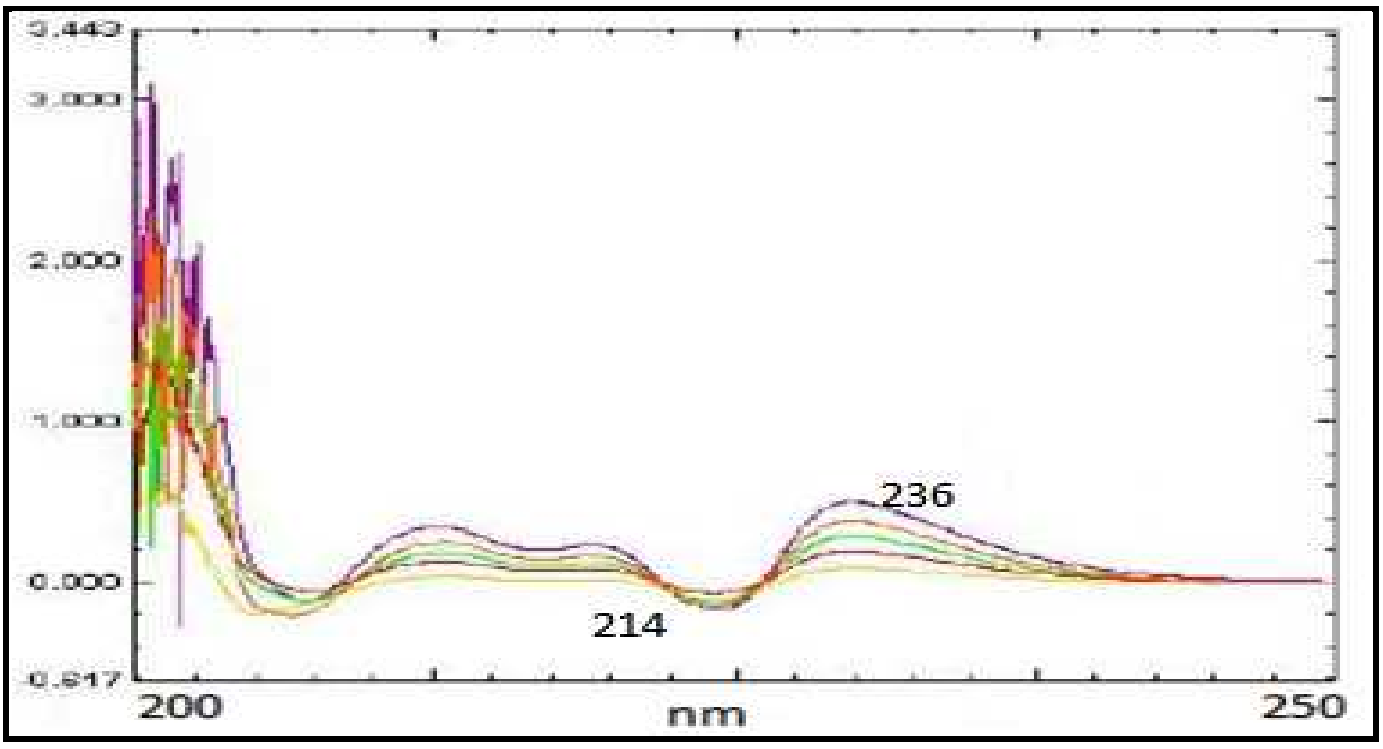

Figure No.3: Overlain spectra for Nelfinavir mesylate in Acidic and Alkaline medium

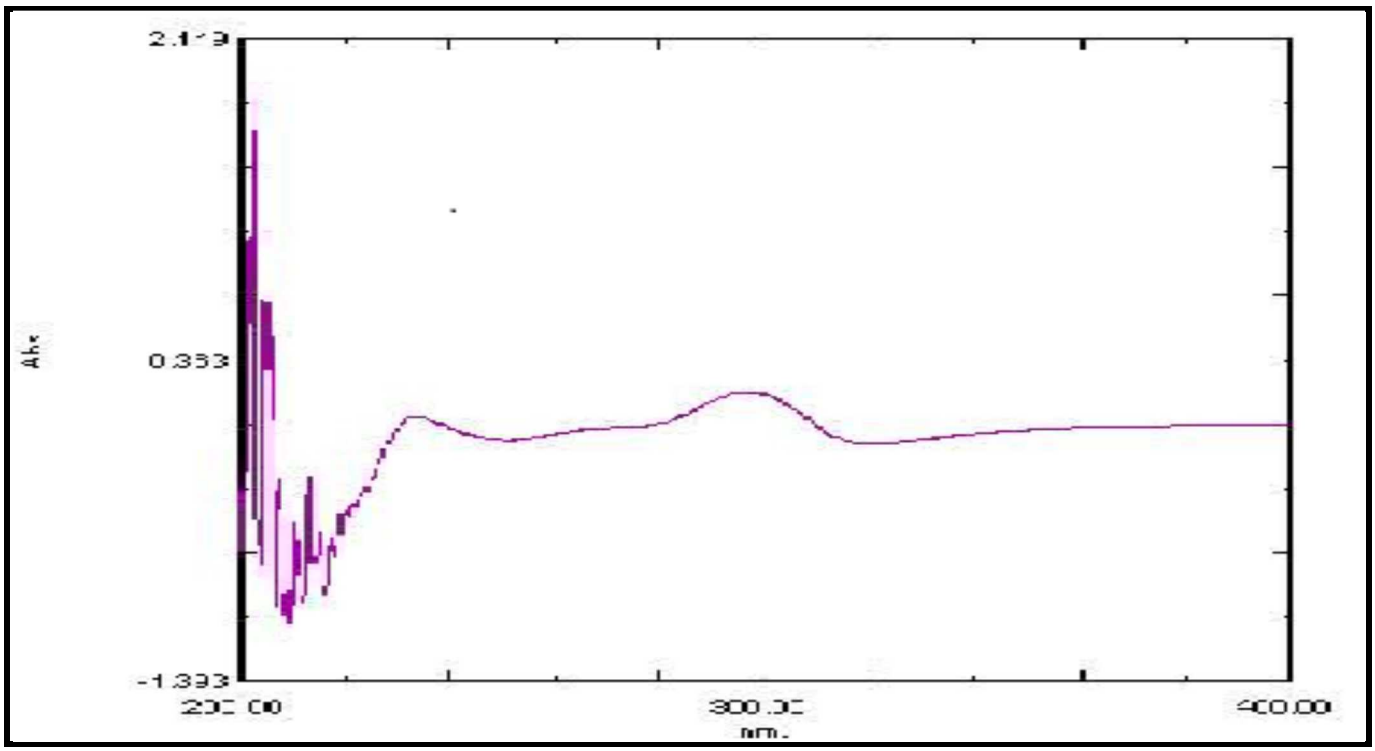

Figure No.4: Assay curve for market formulation (Nelfinavir mesylate tablets)

CONCLUSION

The method is found to be simple, economical, selective and sensitive. The low value of relative standard deviation for repeated measurement indicates that the method is precise. The statistical parameters clearly indicate the reproducibility and accuracy of the method. Analysis of Nelfinavir mesylate in its dosage forms showed no interference from the common excipients and additives. Difference Spectrophotometry by indicating pH of the medium may be recommended for routine and quality control analysis of the investigated drug in tablets.

ACKNOWLEDGEMENT

The authors are sincerely thanks to Creative Educational Society's College of Pharmacy, Kurnool, Andhra Pradesh, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Madhu Kumar G. *et al*. *In* Development and validation of differential spectrophotometric method for determination of nelfinavir mesylate in tablet dosage form, *International Journal of Research in Pharmaceutical and Nano Sciences*, 2(3), 2013, 358-364.