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ESTIMATION OF NELFINAVIR MESYLATE IN PHARMACEUTICAL DOSAGE FORM BY SPECTROFLUORO-PHOTOMETRY

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ABSTRACT

A new specific, selective and inexpensive spectrofluorophotometric method has been developed for determination of Nelfinavir mesylate in bulk drug and its dosage form. Fluorescence spectrum of Nelfinavir mesylate in methanol showed excitation wavelength at 253 nm and emission wavelength at 508 nm. The calibration curve for Nelfinavir mesylate was found to be linear over the concentration range of 100-225 ng/mL with correlation coefficient of 0.988 for bulk drug. Limit of detection and limit of quantification were found to be 8.42 ng/mL and 28.09 ng/mL respectively. Method was validated as per ICH guidelines and found to be suitable for estimation of Nelfinavir from bulk drug and its pharmaceutical dosage form.

INTRODUCTION: Protease inhibitors represent potent drugs for a sufficient drug exposure to maintain anti-viral treatment of HIV disease. Nelfinavir mesylate (NM) is chemically (3S, 4aS, 8aS)-N-*tert*-Butyl-2-[(2R, 3R)-3-(3, 2-cresotamido)-2-hydroxy-4-(phenyl thio) butyl] decahydro-3-isoquinolinecarboxamide mono methane sulfonate (salt) (Figure 1) used as protease inhibitor It is slightly soluble in water and highly lipophilic in nature [1].

NM is official in IP 2010 [2]. Various methods have been reported for quantitative and qualitative estimation of NM in plasma; bulk drug and pharmaceutical formulation are UV, HPLC, ion-pair chromatography, LC/MS etc [3-6].

Spectrofluorophotometry methods are highly sensitive, selective and cost-effective than all other advanced methods available, the present article discusses the development and validation of spectrofluorophotometry method for determination of NM.

The objective of this study was to develop accurate, precise, sensitive, selective, reproducible and quick spectrofluorophotometry methods for determination of NM.

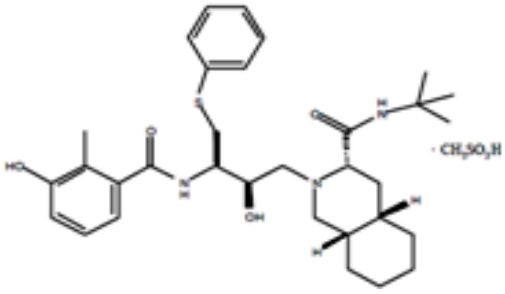

FIGURE 1: STRUCTURE OF NELFINAVIR MESYLATE

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MATERIALS AND METHOD: A Shimadzu Spectrofluorophotometer (RF-5301 PC having software RFCP), equipped with a 1 cm fluorescence free quartz cell having four transparent side was used for all spectral and fluorescence measurements. Glassware used for each procedure were soaked overnight in a chromic mixture ($K_2Cr_2O_7$ + conc. H_2SO_4), rinsed thoroughly with double distilled water and dried in dust-free air. Whatman filter paper No. 42 was used to filter the solution of different formulations to separate them from the solvent immiscible formulation excipients.

Nelfinavir mesylate was obtained as a gift sample from Cipla Ltd (Goa, India). Methanol (Analytical grade) was purchased from Allied Chemicals Corporation, Vadodara, India. Film coated tablets of NM (Nelvir® (250 mg), Cipla) and (Nelfin® (625 mg), Hetero healthcare Ltd., Hyderabad) were procured from local drug store.

1. **Procedure for Standard drug NM:** For the preparation of standard drug solution, NM (50 mg) was accurately weighed and dissolved in 50 ml of methanol to give a stock solution of 1 mg/mL. It was further diluted with methanol to produce 10µg/mL of NM. The aliquotes ranging from 1-2.25 ml were taken with the help of micropipette to prepare a series of standard solutions (100-225 ng/mL) for calibration curve. All the solutions and working standard solution of NM were protected from light by wrapping in aluminum foil.

2. **Procedure for Dosage Form (Tablets):** Twenty tablets were weighed, powdered and tablet powder equivalent to 50 mg of NM was taken and stirred with 20 mL of methanol using magnetic stirrer for 30 min at room temperature. Then it was filtered through Whatman filter paper No 42 into 50 mL volumetric flask. Filter paper was rinsed thrice with 2 mL of methanol and the solution was diluted to 50 mL with methanol to give a stock solution of 1 mg/mL. Then 1 ml of the stock solution was diluted with methanol to produce 10µg/mL of the drug. From above solution three suitable aliquots (1 mL, 1.5 mL and 2 mL) were taken and diluted to 10 mL with methanol to prepare the sample solutions 100 ng/mL (low), 150 ng/mL (middle) and 200 ng/mL (high) in the range of calibration curve.

Assay Procedure: Standard solution (10 µg/mL) of NM was scanned in the range of 200-350 nm for determination of excitation wavelength and it was found to be 253 nm. Same solution was scanned for determination of emission wavelength in the range of 253-800 nm taking 253 nm as excitation wavelength.

RESULT AND DISCUSSION: The relative fluorescence intensity of all the standard solutions was measured in the range of 253-800 nm and the emission wavelength was found to be 508 nm. The emission spectrum at 253 nm is shown in **Figure 2**.

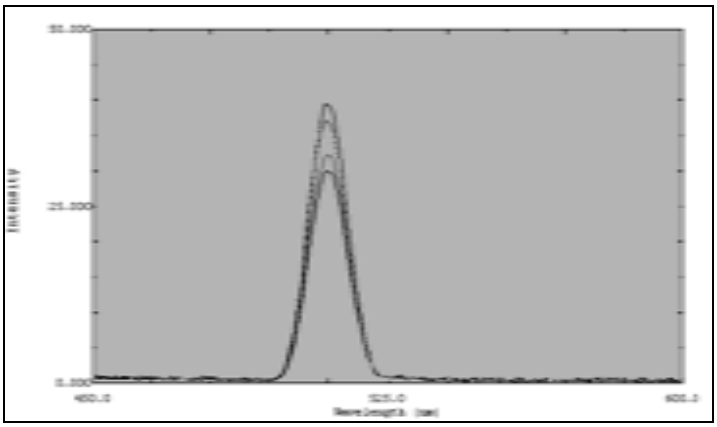

FIGURE 2: EMISSION FLUORESCENCE SPECTRA OF NM AT 253 nm

The developed method was then validated as per ICH guidelines[7] for analytical parameters such as accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). Inter-day and Intra-day RSD were also found out to ascertain precision and accuracy of the developed method. The validation parameters are summarized in **Table 1**.

TABLE 1: OPTICAL CHARACTERISTICS AND ANALYTICAL DATA

Parameters	Observations
Excitation wavelength, $_{Exi\ (nm)}$	253
Emission wavelength ,$_{Emi(nm)}$	508
Linearity range (ng/mL)	100-225 ng
Regression equation	y = 0.095 x + 19.02
Slope(b)	0.095
Intercept(c)	19.02
Coefficient for determination (r^2)	0.988
Limit of Detection, LOD (ng/mL)	8.42 ng/mL
Limit of Quantification, LOQ (ng/mL)	28.09 ng/mL
Inter-day % RSD	0.86%

[a]Y =a+bX, Where X is the concentration (ng/mL).

Linearity: The calibration curve was prepared by plotting concentration against fluorescence intensity. The calibration curve for NM showed linearity in the concentration range of 100-225 ng/mL as shown in **Figure 3**.

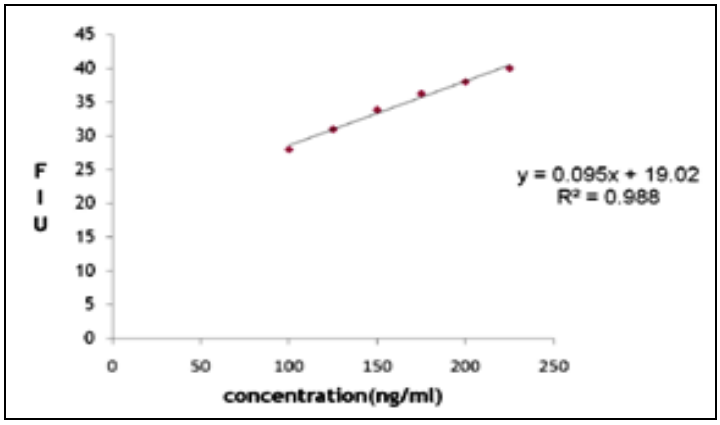

FIGURE 3: CALIBRATION PLOT OF NEFLINAVIR MESYLATE

Calibration curve was repeated five times and RSD of each concentration level was found to be less than 1%, which indicates that method can be used for analysis of bulk drug samples. The correlation coefficient values were highly significant for the method. LOD and LOQ determination: They were determined using following formulas: LOD=3σ/S, LOQ=10σ/S, Where σ is standard deviation of blank, and S is a slope obtained from calibration curve.

Accuracy: Recovery study was carried out by adding a known amount of standard drug to different concentration of sample solutions at three different levels. The total amount of drug was then determined by these method and the amount of added drug found by difference. Result of recovery studies are given in **Table 2**. In this method accuracy was greater than 98 % and RSD did not exceed 2% in any case. The low values of standard deviation and coefficient of variation establish the precision of the proposed method.

Stability of solutions: Standard, stock and working standard of NM did not show significant change in relative fluorescence intensity on storage and hence was stable for up to 6 hr. when wrapped in aluminum foil.

The developed method was applied to marketed formulations for determination of NM content. The results are as shown in **Table 3**.

TABLE 2: RECOVERY STUDY OF COMMERCIAL SAMPLES

Formulation	Standard drug added in final solution (ng/mL)	Drug found* ±SD (ng/mL)	*Recovery (%)
NELVIR (NM-1)	100	100.016 ± 0.86	**100.2**
	150	149.78 ± 0.96	**97.81**
	200	195.66 ± 0.52	**97.85**
NELFIN (NM-2)	100	99.979 ± 0.69	**99.6**
	150	149.854 ± 0.42	**99.9**
	200	195.86 ± 0.16	**97.63**

*Average of three determinations at three levels.

TABLE 3: RESULT OF COMMERCIAL SAMPLES ANALYSIS

Marketed Formulation of NM	Drug/Label Claim (mg/Tablet)	*Amount found (mg)	% Assay
NELVIR (NM-1)	Nelfinvir as free base/ 250	249.81	**99.92**
NELFIN (NM-2)	Nelfinvir as free base / 625	**623.75**	**99.80**

Asterisk (*) denotes mean of five determinations

From the discussion above, it is clear that the developed method is accurate, precise, repeatable, reproducible, linear, quick, inexpensive, sensitive and simple. In absence of any reported method for estimation of NM by spectrofluorophotometry, proposed method is useful in the routine quality control analysis of bulk drug and pharmaceutical formulation.

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