Spectrophometric Determination of Nelfinavir Mesylate

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Abstract: Two new simple, sensitive, rapid and economical Spectrophotometric Methods (A and B) have been developed for the determination of Nelfinavir Mesylate in pharmaceutical bulk and tablet dosage form. The method A is based on the reaction of Nelfinavir with ferric chloride, potassium ferricyanide and hydrochloric acid to form a bluish green colored chromogen. The Method B is based on the formation of blood red colored chromogen with Ferric chloride and 1,10-phenanthroline. The absorbances of the chromogen were measured at their respective wavelength of maximum absorbance against the corresponding reagent blank. The proposed methods have been successfully applied to the analysis of the bulk drug and its tablet dosage form. The methods have been statistically evaluated and were found to be precise and accurate.

Introduction

Nelfinavir Mesylate [1] is a novel HIV-1 protease inhibitor, with a chemical name (3S, 4aS, 8aS)-N- (1,1-Dimethylethyl) decahydro-2- [(2R, 3R)-2-hydroxy-3- [(3-hydroxy-2-methyl benzoyl) amino]–4-(phenylthio) butyl]-3-isoquinolinecarboxamide methanesulfonate .It is an antiretroviral drug that acts by binding reversibly to HIV protease thereby preventing cleavage of the viral precursor polyproteins. It is official in Martindale[2]-The Extra

pharmacopoeia. Literature survey reveals many Chromatographic methods[3-10] for the determination of Nelfinavir in biological fluids and in combination with other antivirals and very few Spectrophotometric methods [11-13] only. Therefore the need for fast, low cost and selective method is obvious especially for routine Quality Control analysis of pharmaceutical formulation.
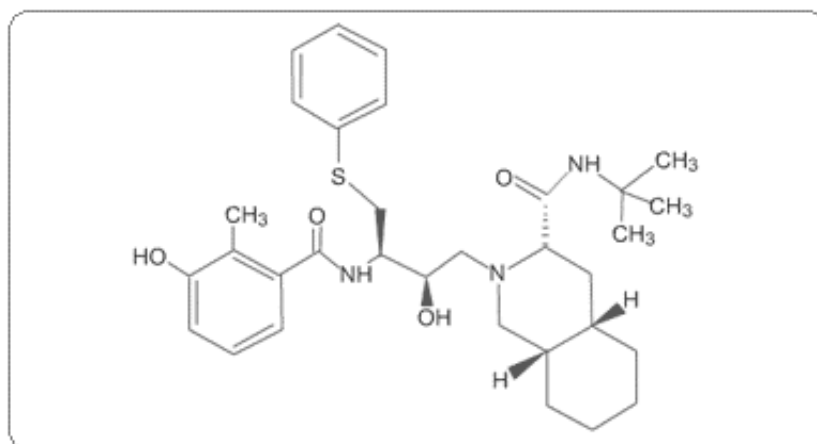
Structure of Nelfinavir

Experimental

Instrument

Elico double beam Ultraviolet-Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

Reagents

All the chemical used were of analytical reagent grade. All the solutions were freshly prepared with distilled water. For Method A aqueous solutions of Ferric chloride (0.3% w/v), potassium ferricyanide (0.1% w//v) and hydrochloric acid (1 N) were prepared. The reagents used in Method B were aqueous solution of Ferric chloride (0.003 M), 1,10-phenanthroline (0.02 M) and o-phosphoric acid (0.2 M).

Procedure

Standard stock solution: A standard stock solution containing 1 mg/ml was prepared by dissolving 100 mg of Nelfinavir Mesylate in 100 ml of methanol. From this, a working standard solution containing 80 µg/ml (Method A) and 50 µg/ml (Method B) were prepared with methanol.

Method A

Into a series of graduated 10 ml test tubes, 0.5-1.5 ml (1 ml=80µg/ml) of working standard drug solution was pipetted separately and to each test tube 0.5 ml of 0.3% w/v ferric chloride solution was added and shaken for 2 min. Then 0.5 ml of 0.1% w/v potassium ferricyanide was added and shaken for 2 min. To this solution, 0.5 ml of 1N hydrochloric acid was added and shaken for 2 min. Then final volume was made up to 10 ml with distilled water. The absorbance of the bluish green color developed was measured at 720 nm against reagent

blank within 25 mts. The sample solution was also treated in the similar manner. The amount of drug in the sample was computed from Beer-Lambert plot.

Method B

Aliquots of standard solutions containing 0.1-2.0 ml (1ml= 50µg/ml) were transferred into series of 10 ml graduated test tubes, 3 ml of ferric chloride (0.003 M) and 1.5 ml of 1,10-phenanthroline (0.02 M) were added to each test tube. The test tubes were then heated on water bath for 15 min at a temperature of 70^0c, then cooled to room temperature and 1 ml of o-phosphoric acid (0.2 M) was added to each test tube and the total volume was brought to 10 ml with distilled water. The absorbance of the blood red colored species was measured at 510 nm against reagent blank. The amount of Nelfinavir Mesylate present in the sample solution was computed from its calibration curve.

Preparation of sample solution

Tablets containing Nelfinavir Mesylate were successfully analyzed by the proposed methods: Twenty tablets of Nelfinavir Mesylate (NELFIN, 250 mg, Genix Pharma) were accurately weighed and powdered. Tablet powder equivalent to 100 mg of Nelfinavir was dissolved in 50 ml of methanol and filtered and washed with methanol, the filtrate and washings were combined and the final volume was made to 100 ml with methanol. The solution was suitably diluted and analyzed as given under the assay procedure for bulk samples. The results are represented in Table 1. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Nelfinavir, by the proposed methods

Recovery Studies

To ensure the accuracy and reproducibility of the results obtained, recovery experiments were performed by adding known amounts of pure drug to the previously analysed formulated samples and these samples were reanalyzed by the proposed method. The percentage recoveries thus obtained were given in Table 1.

Table 1. Assay of Nelfinavir Mesylate in Tablets.

Sample (Tablet)	Labeled Amount (mg)	Amount Obtained (mg)* by proposed method		** % Recovery by the proposed method	
		Method A.	Method B	Method A.	Method B
1	250	253.30	252.47	100.5%	100.9%
2	250	253.10	252.31	99.8%	100.9%
3	250	253.00	252.10	100.6%	100.8%

*Average of three determinations.
** After spiking the sample.

Results and Discussion

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen. The effect of temperature of the reaction, quantity, concentration and addition of various reagents were studied, optimized after several experiments and incorporated in the procedure.

In the Method A, the drug Nelfinavir reduces ferric chloride to ferrous ions, which in turn couples with reagents having divalent iron like potassium ferricyanide to form bluish

green colored potassium ferro ferrous complex. The absorption spectrum of the colored solution showed maximum absorption at 720 nm. The bluish green color was found to be stable for 25 min only. In the Method B Nelfinavir Mesylate reduces ferric chloride to ferrous form, which forms complex with 1.10-phenanthroline to yield blood red colored chromogen. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 2**.**

The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in Table 2.

Table 2**.** Optical Characteristics and Precision Data**.**

	Method A	Method B
λ_{max} (nano meters)	720	510
Beer's law limits (micrograms/ml)	4 – 12	0.5-10
Molar absorptivity (l/mol.cm)	3.46×10^3	0.6×10^3
Sandell's sensitivity (micrograms/cm^2/0.001 absorbance unit)	0.192	0.106
Regression Equation* (Y)		
Slope (m)	0.008	0.007
Intercept (c)	-0.247	0.018
Correlation Coefficient(r)	0.9998	0.998
Precision (% Relative Standard Deviation)	0.923	0.2986
Standard error of mean	0.0049	0.0288

*Y=mx+c, where X is the concentration in micrograms/ml and Y is absorbance unit.

Conclusions

The proposed methods are economical, simple, sensitive and accurate for the routine estimation of Nelfinavir Mesylate in bulk as well as in tablet form.

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