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RP-HPLC Estimation of Nelfinavir Mesylate in Bulk Drug and Pharmaceutical Formulation

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A simple, rapid and reproducible high performance reversed phase liquid chromatographic method has been developed for the estimation of nelfinavir in bulk drug sample and pharmaceutical dosage forms using kromosil C_{18} column. The mobile phase consists of potassium dihydrogen phosphate and acetonitrile in the ratio of 30:70 v/v, respectively and was pumped at rate of 1.3 mL/min at 25 °C. The detection was carried out at 220 nm and the calibration curve was linear in the range of 5 to 30 µg/mL. The method was statistically validated for its linearity, precision and accuracy. The intra-day and inter-day variation was found to be less than 1 % showing high precision of the assay method. Due to its simplicity, rapidness, high precision and accuracy, the proposed RP-HPLC method may be used for determining nelfinavir mesylate in bulk drug sample or in pharmaceutical formulations.

Key Words: Nelfinavir mesylate, RP-HPLC.

INTRODUCTION

Nelfinavir mesylate[1] is a novel HIV-1 protease inhibitor with a chemical name N-(1,1-dimethylethyl)decahydro-2-[(2R 3R)-2-hydroxy-3-[(3-hydroxy-2-methyl benzoyl)amino]-4-(phenylthio)butyl]-3-isoquinoline carboxamide methane sulfonate. 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 literature survey reveals many chromatographic methods[3-7] for determination of nelfinavir in biological fluids and in combination with other antiviral and few spectrophotometric methods have been reported[8-10]. Therefore, the need for a fast, low cost and selective method is obvious especially for routine quality control analysis of pharmaceutical formulations. The aim of this study is to develop a simple, rapid, precise and accurate RP-HPLC method for the determination of nelfinavir mesylate in bulk drug sample or in pharmaceutical dosage forms.

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EXPERIMENTAL

Quantitative HPLC was performed on a isocratic high pressure liquid chromatograph (Shimadzu HPLC class VP series) with LC-10 AT VP pump, variable wavelength programmable UV/Vis detector SPD-10 A VP, CTO-10 AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard)^{TM}, LC-18, 2 cm, supelco Inco, Bellefonte, PA and RPC-18 column kromsil (250 × 4.6 mm) was used. The HPLC system was equipped with the software 'class-VP series version 6.01 (Shimadzu)'.

Pure sample of nelfinavir mesylate was obtained as gift sample from Alkem Laboratories, Ltd Mumbai, India. Acetonitrile, methanol and water (HPLC grade Qualigens), potassium dihydrogen phosphate (AR grade) purchased from Merck Limited, Mumbai (India). The commercial available nelfinavir tablet claimed to contain 250 mg of drug were procured from local market.

Chromatographic condition: The content of the mobile phase were potassium dihydrogen phosphate and acetonitrile in the ratio of 30:70. 0.01 M potassium dihydrogen phosphate was prepared by dissolving 1.36 g in 1000 mL of water (HPLC grade). The contents of the mobile phase were filtered before use through 0.45 μm membrane filter, degassed with helium, purge for 15 min and pumped from the respective solvent reservoirs to the column at the flow rate of 1.3 mL/min which yielded a column back pressure of 110-118 kg/cm^2. The run time was set at 15 min and the column temperature was maintained at 25 °C. The volume of injection loop was 10 μL. Prior to the injection of drug solutions, the column was equilibrated for at least 0.5 h with mobile phase flowing through the system. The elements were monitored at 220 nm and the data were acquired, stored and analyzed with software 'Spinchrom CFR (Shimadzu)'.

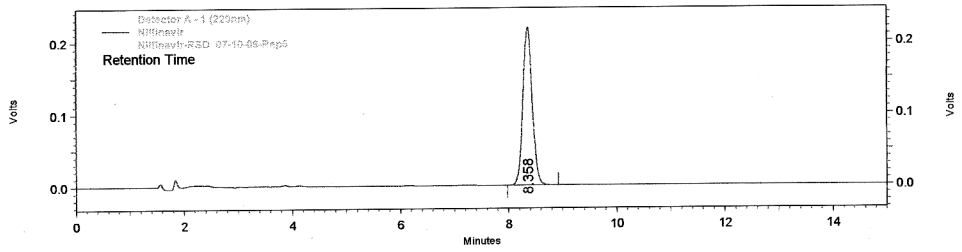
Procedure: About 100 mg of nelfinavir mesylate was accurately weighed and dissolved in methanol so as to give 1 mg/mL solution. Subsequent dilution of this solution was made with mobile phase to get concentration of 5 to 30 μg/mL of nelfinavir mesylate. The standard solution prepared as above was injected six times into a column at a flow rate of 1.3 mL/min. The peak area of drug concentration was calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of nelfinavir mesylate in tablet dosage form. Nelfinavir mesylate solutions containing 20 and 40 μg/mL were subjected to the proposed RP-HPLC analysis for finding out intra and interday variations. The recovery studies were carried out by adding known amount of nelfinavir mesylate to pre-analyzed sample and subjecting them to the proposed RP-HPLC method.

Estimation of nelfinavir mesylate: Ten tablets each containing 250 mg were weighed and powdered. An accurately weighed portion of the powder equivalent to 100 mg of nelfinavir mesylate was transferred to a 100 mL volumetric flask containing 50 mL of methanol. The contents of the flask were sonicated for 15 min to dissolve nelfinavir mesylate and made upto. The volume with methanol and the

resulting mixture was filtered through 0.45 µm filter. Subsequent dilution of this solution were made with mobile phase to get concentration of 100 µg/mL. 1 mL of this solution was added to 10 mL volumetric flask and made up to the volume with mobile phase. This solution (10 µL) was injected six times into the column. The mean values of peak areas of six such determinations were calculated and the drug content in the tablet was quantified using the regression equation obtained as above. The same procedure was followed for the estimation of nelfinavir mesylate in other commercially available tablet dosage forms.

R.S.D. of Nelfinavir

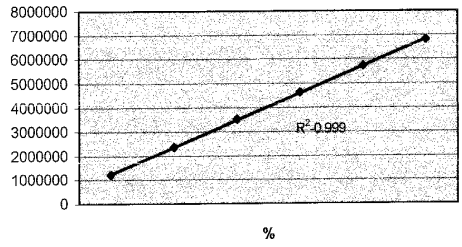
S.No.	µg/mL	Area	
1	10	2611606	
2	10	2615251	S.D = 8179.26
3	10	2610658	Mean = 2606805.16
4	10	2609702	R.S.D. = 0.3137
5	10	2599719	
6	10	2593895	



Chromatogram of nelfinavir mesylate

$R^2 = 0.999$

On X-axis-µg	On Y-axis-Area
5	1190065
10	2343780
15	3498817
20	4604130
25	5709727
30	6789543



RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive drug, precise and accurate RP-HPLC method for the analysis of nelfinavir mesylate in bulk sample or pharmaceutical dosage forms. The column pressure varied from 110-118 kg/cm^2. The retention time for nelfinavir mesylate was found to be 8.358 min for a run period of 14 min. Each of samples was injected six times and the same retention times were observed in all cases. The peak area of different concentration set up as above were caculated

and average value for six such determinations are shown in Table-1. The peak area for drug solution was reproducible as indicated by low coefficient of variation (0.313). A good linear relationship (0.9998) was observed between the concentration of nelfinavir mesylate and the respective peak areas. The calibration graph was found to be Y = 239317.93, where Y is the peak area and C is the concentration of nelfinavir mesylate in the range of 2.5 to 30 µg/mL, when the nelfinavir mesylate solution containing 20 and 40 µg/mL were analyzed by the proposed RP-HPLC method for the finding out the intra and inter day variation, a low co-efficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise. The amount of drug from preanalyzed sample containing known amount of drug are shown in (Table-3). About 99.26 % nelfinavir mesylate could be recovered from the pre-analyzed sample indicating the high accuracy of the proposed HPLC method. The drug content in the tablet was quantified using the proposed analytical method. The mean content of nelfinavir mesylate in two different brands of tablet dosage forms is shown in Table-4.

TABLE-1
CALIBRATION OF THE RP-HPLC METHOD FOR THE ESTIMATION OF NELFINAVIR MESYLATE

Concentration of nelfinavir (µg/mL)	Peak area (n=6)	Concentration of nelfinavir (µg/mL)	Peak area (n=6)
2.5	595032	22.5	5179646
5.0	1190065	25.0	5709727
7.5	1785097	27.5	6280699
10.0	2343780	30.0	6789543
12.5	2929725	Regression equation (y*)	
15.0	3498817	Slope (b)	225473.31
17.5	4081953	Intercept (a)	85076.515
20.0	4604130	Correlation coefficient (r)	0.9998

*y = bC+ a; Y is peak area and C is the concentration of nelfinavir mesylate in the range of 2.5 to 30 µg/mL.

TABLE-2
INTER AND INTRA-DAY PRECISION FOR NELFINAVIR ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED RP-HPLC METHOD

Concentration of nelfinavir (µg/mL)	Observed concentration of nelfinavir			
	Intra-day		Inter-day	
	Measured conc. (µg/mL) ± SD	% C.V.	Measured conc. (µg/mL) ± SD	% C.V.
20	2211.91	0.0350	1494.97	0.05744
40	975.754	0.0215	147.217	0.00324

The absence of additional peaks indicates no interference of the excipents used in the tablet. The tables were found to contain 98.32 to 99.28 % of the labeled amount. Less than 1 % C.V. indicates the reproducibility of the assay of nelfinavir mesylate in the tablets dosage form.

TABLE-3
EXPERIMENTAL VALUES OBTAINED IN RECOVERY TEST FOR NELFINAVIR MESYLATE TABLETS BY PROPOSED RP-HPLC METHOD

Amount of drug added (µg) to drug solution /powder tablet formulation	Recovery from drug solution		Recovery from powdered tablet formulations	
	Mean (±SD) Amount (µg) Found (n=6)	Mean (±SD) % recovery (n = 6)	Mean (±SD) Amount (µg) Found (n=6)	Mean (±SD) % recovery (n=6)
25	24.58	98.32	24.82	99.28
50	44.63	99.27	49.62	99.26

TABLE-4
MEAN (±SD) AMOUNT OF NELFINAVIR MESYLATE IN TABLET DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Brand of tablet	Labelled amount of drug (mg)	Mean (±SD) amount found (mg) by the proposed method (n=6)	Mean (±SD) % labeled amount (n=6)
Nelvir 250 (Cipla)	250	245.82	98.33
Retronel 250 (Alkem)	250	248.21	99.29

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