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Estimation of Saquinavir Mesylate in Tablet Dosage Form using RP-HPLC

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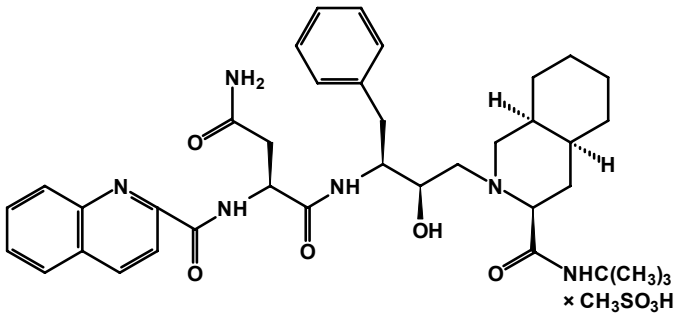
A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of saquinavir mesylate in tablet dosage form. A Sun Fire C18, 250 mm × 4.6 mm i.d, 5 µm partical size, with mobile phase consisting of acetonitrile and 0.03 M potassium dihydrogen phosphate (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 50:50 v/v was used. The flow rate was 1 mL/min and the effluents were monitored at 235 nm. The retention time was 3.98 min. The detector response was linear in the concentration of 20-240 mcg/mL. The respective linear regression equation being Y= 26704.6X + 84052.1. The limit of detection and limit of quantification was 0.004 and 0.012 mcg/mL, respectively. The percentage assay of saquinavir mesylate was 99.87 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of saquinavir mesylate in bulk drug and in its pharmaceutical dosage form.

Key Words: Saquinavir mesylate, RP-HPLC, Estimation and Tablets.

INTRODUCTION

Saquinavir mesylate[1] is a novel HIV-1 protease inhibitor with a chemical name N-*tert*-butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methanesulfonate with m.f. $C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$ and m.w. of 766.96. The molecular weight of the free base is 670.86. It is an antiretroviral drug that acts by binding reversibly to HIV protease thereby preventing cleavage of the viral precursor polyproteins. Literature survey reveals many chromatographic methods[2-8] for the determination of saquinavir in combination with other antiviral, in biological fluids and only one spectrophotometric method[9] only. So far, no assay procedure has been

reported for the estimation of saquinavir mesylate from pharmaceutical dosage forms. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of saquinavir mesylate in bulk drug samples and in pharmaceutical dosage form.



Structure of saquinavir mesylate

EXPERIMENTAL

Saquinavir mesylate was obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Potassium dihydrogen orthophosphate was of analytical grade and supplied by M/s S.D. Fine Chem Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available saquinavir mesylate tablets (Saquin-500) were procured from local market.

Quantitative HPLC was performed on liquid chromatograph, waters separation 2996, PDA detector module equipped with automatic injector with injection volume 10 µL and 2693 pump. A RP C-18 Sun Fire column (250 mm × 4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions: The contents of the mobile phase were acetonitrile and 0.03 M potassium dihydrogen phosphate (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 50:50 v/v. They were filtered before use through a 0.45 µm membrane filter and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 10 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 0.5 h with the mobile phase flowing through the system. The eluents were monitored at 235 nm.

Preparation of standard stock solution: A standard stock solution of the drug was prepared by dissolving 50 mg of saquinavir mesylate in 50 mL volumetric flask containing 30 mL of diluent (50:50 v/v acetonitrile:water), sonicated for about 15 min and then made up to 50 mL with diluent to get a 1 mg/mL standard stock solution.

Working standard solution: 10 mL of the above stock solution was taken in 50 mL volumetric flask and thereafter made up to 50 mL with diluent to get a concentration of 200 µg/mL.

Preparation of sample solution: 20 Tablets (Saquin-500, Genix Pharma) were weighed and then powdered. A sample of the powdered tablets, equivalent to 50 mg of the active ingredient, was mixed with 25 mL of diluent. The mixture was allowed to stand for 0.5 h with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 µm membrane filter, followed by adding diluent to obtain a stock solution of 1.0 mg/mL. An aliquot of this solution was transferred to a 10 mL volumetric flask and made up to sufficient volume with mobile phase to give an concentration of 200 mcg/mL.

Linearity: Aliquots of standard saquinavir mesylate stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of saquinavir are in the range of 20-240 mcg/mL. Each of these drug solutions (20 µL) was injected three time into the column and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 235 nm and a calibration graph was obtained by plotting peak area *vs.* concentration of saquinavir mesylate.

The plot of peak area of each sample against respective concentration of saquinavir mesylate was found to be linear in the range of 20-240 µg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table-1. The respective linear regression equation being Y= 26704.6X + 84052.1. The regression characteristics, such as slope, intercept, standard deviation on slope (Sa), the standard deviation of the intercept (Sb) and % RSD were calculated for this method and given in Table-1.

TABLE-1
LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Drug	Saquinavir mesylate
Concentration range (mcg/mL)	20-240
Slope (m)	26704.6
Standard deviation on slope (Sm)	2.58×10^{2}
Intercept (b)	84052.1
Standard deviation on intercept (Sb)	4.75×10^{4}
Correlation coefficient	0.9999
% RSD	0.13

Assay: 10 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 3.98 min. The amount of drug present per tablet was calculated by comparing the peak

area of the sample solution with that of the standard solution. The data are presented in Table-2.

TABLE-2
RESULTS OF HPLC ASSAY AND RECOVERY STUDIES

Sample	Amount claim (mg/tablet)	%Found by the proposed method	%Recovery*
1	500	99.85	103.37
2	500	100.52	102.00
3	500	99.26	98.37

*Average of three different concentration levels.

Recovery studies: Accuracy was determined by recovery studies of saquinavir mesylate, known amount of standard was added to the preanalyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table-2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of saquinavir mesylate. Parameters that were studied to evaluate the suitability of the system are given in Table-3.

TABLE-3
VALIDATION SUMMARY

Validation parameter	Results
Theoretical plates (N)	7713
Tailing factor	1.36
Retention time (min)	3.98
LOD (mcg/mL)	0.004
LOQ (mcg/mL)	0.012

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) for saquinavir mesylate were found to be 0.004 and 0.012 µg/mL, respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

From the typical chromatogram of saquinavir mesylate, it was found that the retention time was 3.98 min. A mixture of acetonitrile and 0.03 M potassium dihydrogen phosphate (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 50:50 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r = 0.9999)

was observed between the concentration range of 20-240 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of saquinavir mesylate tablets was found to be 99.87 %. From the recovery studies it was found that about 101.24 % of saquinavir mesylate was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of saquinavir mesylate within a short analysis time.

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