Research Article

Spectrofluorimetric Method Development and Validation of Saquinavir Mesylate in Bulk and Tablet Dosage Form

Swathi Naraparaju[1*], Durai Ananda Kumar Thirumoorthy[2], Karuna Devi Barla[1], Durga Pani Kumar Anumolu[3], Prathusha Machavaram[3]

[1]Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Hyderabad-500 090, Telangana, India.
[2]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, SS Nagara, Mysuru, 570 015, Karnataka, India.
[3]Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Hyderabad-500 090, Telangana, India.
***Corresponding author's E-mail:** swa.pharma@gmail.com

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ABSTRACT

A new, simple and affordable spectrofluorimetric method was developed for the quantification of saquinavir mesylate in bulk and marketed formulation. The method was established by measuring the native fluorescence of saquinavir mesylate in acetate buffer pH 4.7 at 438 nm after excitation at 372 nm. Linearity in the response was noticed in the concentration range of 10-50 µg/mL. The method was supported by checking several validation parameters as stated by ICH guidelines. The limit of detection and quantification values (0.907 and 2.75 µg/mL, respectively) demonstrated that the procedure was accurate, precise and reproducible (% relative standard deviation < 2.0). The % saquinavir mesylate in commercial formulation was found to be 98.4, which is in agreement with ICH guidelines. Thus the developed method is simple and can be successfully adopted in routine analysis of saquinavir mesylate in pharmaceutical dosage forms.

Keywords: Saquinavir mesylate, Spectrofluorimetry, Accuracy, Linearity.

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INTRODUCTION

Saquinavir was the first FDA-approved HIV protease inhibitor used in the treatment of AIDS.[1] The chemical name of saquinavir is (2S)-N-[(2S,3R)-4-[(3S)-3-(tert-butylcarbamoyl)-3,4,4a,5,6,7,8,8a-octahydro-1H-iso quinolin-2-yl]-3-hydroxy-1-phenylbutan-2-yl]-2-(quinoline -2-carbonylamino)butane- -diamine[2] and the structure is shown in **Figure 1**.

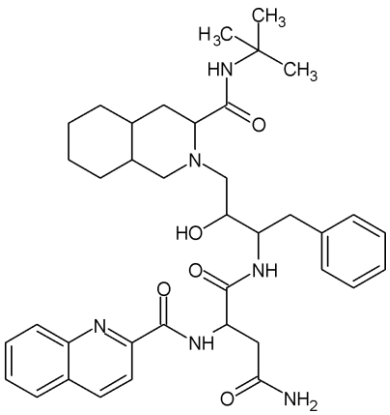

Figure 1: Structure of saquinavir

It is available as saquinavir mesylate under the brand name, Invirase. It is available in capsule and film-coated tablet dosage forms.[3] Saquinavir is used in combination with ritonavir (Norvir) and other antiviral medications to treat HIV.[4] It is a specific inhibitor of HIV-1 and 2 proteases, and also inhibits CYP3A. The most common side effects are circumoral paresthesia, asthenia, diarrhea and nausea.

Various methods have been reported for analysis of saquinavir mesylate, such as extractive colorimetric methods using bromocresol green and Orange-II as reagents and UV spectrophotometric method using methanol as solvent.[5,6] The methods outlined also include RP-HPLC with UV detection[7] and fluorescence detection[8], HPLC with UV detection using CaCo-2 cell monolayers[9] and spectrophotometric method using 20% methanol as solvent.[10]

A few simultaneous estimation methods of saquinavir along with other antiretroviral drugs using HPLC[11] and stability indicating RP-HPLC method using 0.1 M phosphate buffer (pH 3.5): methanol (70:30, v/v) as mobile phase were also reported.[12] However, the chromatographic methods require costly instrumentation, skilled technician and costly solvents. An extensive literature search revealed that there was no simple spectrofluorimetric method for the assessment of saquinavir mesylate to the best of our cognition. Spectrofluorimetry has been playing a vital role in drug quantification due to its appreciable sensitivity and specificity than spectrophotometry using two wavelengths, excitation and emission. [13]

Keeping the above facts in view, a spectrofluorimetric method was attempted for the evaluation of saquinavir mesylate. The method does not involve an extraction step, thus lessen the time and errors involved in the analytical process.

MATERIALS AND METHODS

Materials

Analytically pure saquinavir mesylate was acquired as a gift sample from a reputed pharmaceutical company. The solvents and chemicals used in the study were of analytical grade (Merck, India). The commercial formulation, Invirase tablets (500 mg of saquinavir mesylate per tablet) were procured from a neighbourhood drug store.

Instrumentation

Spectrofluorimeter Model RF-5301PC (Shimadzu, Japan) furnished with a xenon lamp along with 3 mm quartz cells were utilized. Measurement of pH was done using Elico LI 120 pH meter (India). The conventional statistical functions in MS-EXCEL were utilized to compute arithmetic mean (AM), standard deviation (SD) and percentage relative standard deviation (%RSD).

Methods

Preparation of buffer

Acetate buffer of pH 4.7 was prepared by dissolving anhydrous sodium acetate (8.4 g) and glacial acetic acid (3.35 mL) in sufficient distilled water. The pH is then adjusted to 4.7 and the volume was made up with distilled water to produce 1000 mL. [14]

Preparation of standard stock solution of saquinavir mesylate

Saquinavir mesylate stock solution was produced by dissolving 10 mg of the analyte in methanol (10 mL) by ultrasonication (1000 µg/mL). This solution was appropriately diluted to get 10 µg/mL of saquinavir mesylate in acetate buffer pH 4.7 and the same was utilized for finding optimum emission and excitation wavelengths.

Construction of calibration curve

A series of solutions containing saquinavir mesylate in the concentration of 10-50 µg/mL in acetate buffer pH 4.7 were produced by serial dilution of the initial stock solution. The fluorescence intensities of the resulting solutions were recorded at 438 nm after excitation at 372 nm. Relative fluorescence intensities and final concentrations (µg/mL) were plotted on Y- and X-axis, respectively to afford calibration curve.

Analytical method validation

The analytical method was confirmed using different validation parameters, such as linearity, limit of detection, limit of quantification, accuracy and precision as per ICH guidelines. [15, 16]

Linearity

The method linearity was established by preparing a series of solutions containing 10-50 µg/mL concentrations of saquinavir mesylate in acetate buffer pH 4.7. The fluorescence intensities at 438 nm were recorded for triplicate solutions at each concentration. The results were graphed as a calibration curve.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and quantification (LOQ) of the method were determined using samples containing very low concentrations of saquinavir mesylate as per ICH guidelines. The LOD and LOQ were calculated using 3.3*(standard deviation/slope) and 10*(standard deviation/slope), respectively.

Accuracy

The accuracy of the proposed method was determined by computing recoveries of saquinavir mesylate adopting method of standard additions. Tablet powder equivalent to 10 µg/mL of saquinavir mesylate was taken in 10 mL volumetric flask. To this 80, 100 and 120% of standard solutions of saquinavir mesylate were added and made the volume up to mark with acetate buffer pH 4.7. The fluorescence intensities of the emerged solutions were resolute at emission wavelength, 438 nm.

Precision

The repeatability or intra-day precision of the present method was set by estimating the corresponding response three times on the same day for three distinct concentrations of saquinavir mesylate (10, 20 and 30 µg/mL). The intermediate or inter-day precision was determined by estimating selected concentrations (10, 20 and 30 µg/mL) response in triplicate on three different days over a week period. The results of both the studies were expressed as percentage relative standard deviation (%RSD).

Assay of saquinavir mesylate

Twenty tablets of Invirase formulation containing 500 mg of saquinavir mesylate were weighed precisely and powdered. A quantity of powder analogous to 10 mg of saquinavir mesylate was dissolved in methanol (by ultrasonication) and the volume made up to 10 mL. The resulting solution was filtered via Whatman filter paper. An aliquot of the clear filtrate was suitably diluted with acetate buffer pH 4.7 to get 20 µg/mL of saquinavir mesylate. The fluorescence intensity of the later solution was measured at λ_{em} 438 nm. The amount of saquinavir mesylate was determined by substituting responses into equations of the straight line representing the calibration curve, with correction for dilution.

RESULTS AND DISCUSSIONS

Development and optimization of Analytical method

The solubility of the saquinavir mesylate was initially studied in various organic solvents (methanol, ethanol, dimethyl formamide, dimethyl sulfoxide), acids (hydrochloric acid, glacial acetic acid), buffers (phosphate buffers, borate buffers and acetate buffers) and surfactants (CTAB, Sodium lauryl sulphate and Tween-60). Solubility of the analyte was observed only in methanol and acetate buffer pH 4.7. Hence the initial analyte solution was made with methanol (1000 µg/mL) and later acetate buffer pH 4.7 was used for dilution (100 and 10 µg/mL). The fluorescence absorption of the later solution (10 µg/mL) was measured using acetate buffer pH 4.7 as blank. The drug exhibited intense fluorescence at 438 nm following excitation at 372 nm (**Figure 2**).

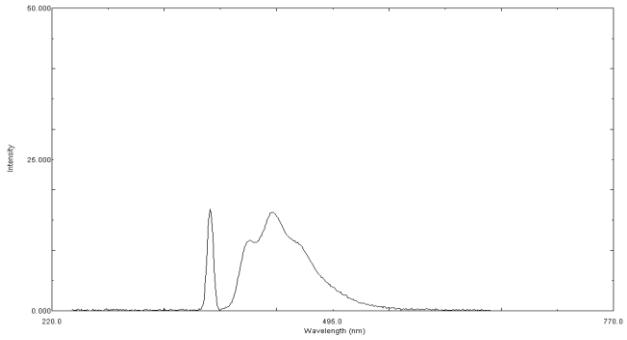

Figure 2: Emission spectrum of saquinavir mesylate in acetate buffer pH 4.7

A linear calibration curve for the method was obtained in the concentration range 10-50 µg/mL for saquinavir mesylate at 438 nm. The calibration curve was shown in **Figure 3**.

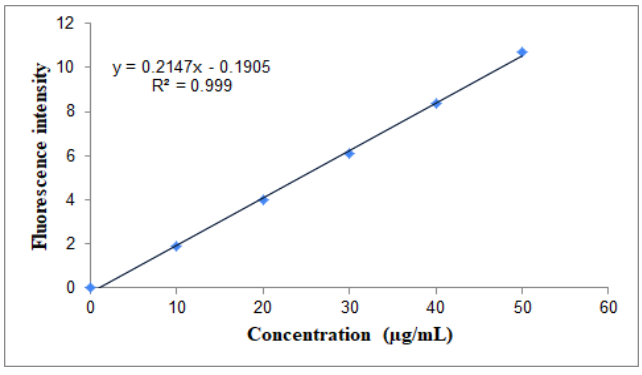

Figure 3: Calibration curve of saquinavir mesylate

The results revealed a linear relationship between concentrations of saquinavir mesylate and the relative fluorescence intensities. From the linear regression analysis correlation coefficient value (r^2) of 0.999 indicated the same.

Method validation

Linearity

The linearity was assessed by the least square regression method by measuring the responses of different concentrations of saquinavir mesylate at emission wavelength 438 nm and the results were shown in **Figure 3**. The relationship between saquinavir mesylate concentration and corresponding fluorescence intensity was found to be linear over the concentration range of 10-50 µg/mL with a r^2 of 0.999. The regression equation obtained was relative fluorescence intensity = 0.2147x - 0.1905.

Limit of detection (LOD) and Limit of Quantification (LOQ)

Responsiveness of the method in terms of LOD and LOQ was determined by utilizing the formulae given in the experiment. The method resulted in 0.907 and 2.75 µg/mL of LOD and LOQ, respectively. The summary of the system suitability parameters of the optimized method were given in **Table 1**.

Table 1: System suitability parameters of the method

Parameter	Values
Excitation wavelength (nm)	372
Emission wavelength (nm)	438
Linearity Range (µg/mL)	10-50
Correlation coefficient (r^2)	0.999
Slope (m)	0.2147
Intercept (c)	-0.1905
Standard deviation	0.059
Regression equation	Y = 0.2147x - 0.1905
Limit of Detection (µg/mL)	0.907
Limit of Quantification (µg/mL)	2.75

Accuracy (recovery studies)

Three distinct levels, 80, 100 and 120% of standards (in triplicate) were spiked to commercial tablet powder to determine accuracy of the proposed method. Mean of %recoveries and %RSD of the same were calculated and reported in **Table 2**. The %recoveries were found to be 98.33, 99.0 and 101.36, respectively for the three levels. The results were found to be satisfactory, which was indicated by the %RSD< 2.0.

Table 2: Accuracy data of the proposed method

S. No.	Amount added (mg)	Amount recovered (mg) (AM ± SD) [a]	% Recovery	%RSD [b]
1	18	17.7±0.18	98.33	1.02
2	20	19.8±0.12	99.00	0.61
3	22	22.3±0.21	101.36	0.94

AM: Arithmetic mean; SD: Standard deviation; RSD: Relative standard deviation; [a] Mean of three determinations; [b] Acceptance Criteria: %RSD < 2.0.

Table 3: Precision data of the proposed method

Concentration (µg/mL)	Intra-day Amount found (AM ± SD) [a]	%RSD [b]	Inter-day Amount found (AM ± SD) [a]	%RSD [b]
10	9.92±0.101	1.02	9.77±0.153	1.57
20	19.81±0.221	1.12	20.12±0.102	0.51
30	30.24±0.472	1.56	29.83±0.352	1.18

AM: Arithmetic mean; SD: Standard deviation; RSD: Relative standard deviation; [a] Mean of three determinations; [b] Acceptance Criteria: %RSD < 2.0.

Table 4: Analysis of saquinavir mesylate in tablets

Drug	Brand Name	Label claim (mg)	Amount found (mg) (AM ± SD)[a]	%Assay	%RSD [b]
Saquinavir mesylate	Invirase	500	492±2.341	98.4	0.48

AM: Arithmetic mean; SD: Standard deviation; RSD: Relative standard deviation; [a] Mean of three determinations; [b] Acceptance Criteria: %RSD < 2.0.

Precision

Triplicate samples of three dissimilar concentrations containing 10, 20 and 30 µg/mL of saquinavir mesylate were utilized for ascertaining the intra- and inter-day variability. Results of these studies were provided in **Table 3**. The %RSD values were found to be < 2.0, indicating good precision of the method.

Assay of saquinavir mesylate in commercial tablets

The contemplated method was successfully applied to estimate saquinavir mesylate in commercial tablets. The amount of saquinavir mesylate in the formulation was found to be 492±2.341 mg and the %assay was 98.4 (**Table 4**), which is in the acceptance range of 98.0-101.0% for saquinavir mesylate as per ICH guidelines. The % RSD less than 2.0 indicated the reliability of the present method.

CONCLUSION

A simple and extraction-free spectrofluorimetric method was established for the quantification of saquinavir mesylate using acetate buffer pH 4.7. When the method was validated as per ICH guidelines, it was proven to be accurate and precise. The content of saquinavir mesylate in marketed tablets was estimated and the %RSD was found to be less than 2.0. Based on above results, we conclude that the developed spectrofluorimetric method could be routinely adopted in the quality testing of saquinavir mesylate in pharmaceutical dosage forms.

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