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Extractive colorimetric method for the determination of saquinavir mesylate in dosage form

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ABSTRACT

Two simple, economical, precise, reliable and reproducible visible spectrophotometric methods (A and B) have been developed for the estimation of Saquinavir mesylate in bulk as well as in tablet formulation. The developed methods A and B are based on the formation of ion pair complexes of the drug with Bromocresol green (BCG) and Orange-II extractable into chloroform, which shows absorbance maxima at 435 nm and 486 nm respectively. The absorbance-concentration plot is linear over the range of 2.0-25 mcg/mL for method A and 5- 25 mg/mL for method B. The molar absorptivity, Sandell's sensitivity, Ringbom optimum concentration ranges for the above complexes were calculated and evaluated at their maximum wavelengths. The different experimental parameters affecting the development and stability were studied carefully and optimized. Results of analysis for all the methods were validated statistically and by recovery studies.

Key words: Saquinavir mesylate, bromocresol green, Orange-II, Ultraviolet-Visible double beam spectrophotometer.

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 a molecular formula $C_{38}H_{50}N_6O_5 \bullet CH_4O_3S$ and a molecular weight of 766.96. 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 visible spectrophotometric procedure has been reported for the estimation of Saquinavir Mesylate from pharmaceutical dosage forms. The aim of the study was to develop simple, precise and accurate visible extractive spectrophotometric method for the estimation of Saquinavir mesylate in bulk drug samples and in pharmaceutical dosage form.

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 chemicals used were of analytical reagent grade, and all the solutions were freshly prepared in doubly distilled water. Acid phthalate buffer pH 2.4 was prepared as per I.P.

´ Bromocresol green (0.5% w/v)-500 mg is weighed accurately and dissolved in 0.74 ml

of 0.1N Sodium hydroxide and 20 ml of methanol. After the solution is mixed thoroughly, it is made up to 100 ml with distilled water.

- Orange-II (0.2% w/v)- 100 mg is weighed accurately and dissolved in 50 ml of distilled water.
- Chloroform AR grade

Procedure

Standard stock solution: A standard stock solution containing 1mg/mL was prepared by dissolving 100 mg of Saquinavir mesylate in 100 mL of methanol. From this, a working standard solution containing 50 mcg/mL were prepared with methanol.

Assay procedure

Method A

Aliquots of the drug solution of Saquinavir Mesylate 0.4-5.0 ml (50 mcg/mL) are taken and transferred into a series of 125 mL of separating funnel. To each funnel 2 ml of BCG reagent and 2 ml of phthalate buffer was added. Reaction mixture was shaken gently for 5 min. Then 10 ml of chloroform was added to each of them. The contents are shaken thoroughly for 5 min. and allowed to stand, so as to separate the aqueous and chloroform layer. Colored chloroform layer was separated out and absorbance was measured at 435 nm against reagent blank. Calibration curve was prepared from absorbance values so obtained

Method B

Aliquots of the drug solution of Saquinavir Mesylate 1.0- 5.0 ml (50 mcg/mL) are taken and transferred into a series of 125 mL of separating funnel. To each funnel 2 mL of Orange-II reagent and 4 ml of phthalate buffer was added. Reaction mixture was shaken gently for 2 min. Then 10 mL of chloroform was added to each of them. The contents are shaken thoroughly for 5 min and allowed to stand, so as to separate the aqueous and chloroform layer. Colored chloroform layer was separated out and absorbance was measured at 486 nm against reagent blank. Calibration curve was prepared from absorbance values so obtained.

Preparation of sample solution

Tablets containing Saquinavir mesylate were successfully analyzed by the proposed methods. Twenty tablets of Saquinavir mesylate (Saquin-500) were accurately weighed and powdered. Tablet powder equivalent to 100 mg of Saquinavir Mesylate 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 2. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Saquinavir Mesylate, by the proposed methods.

Recovery studies

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analysed formulated samples and these samples were reanalyzed by the proposed method performed recovery experiments. The percentage recoveries thus obtained were given in Table 2.

RESULTS AND DISCUSSIONS

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes are present mainly in anionic form in acidic medium. So when treated with an acid dye such as BCG (Method A) and Orange-II (Method B), a yellow orange ion pair complex is formed, which is extracted using chloroform. The absorption spectra of the ion pair complexes formed were measured at 350-800 nm against reagent blank solution. The developed methods were applied to dosage forms and the obtained results were evaluated statistically. The Student's.

T-test and F-test values for the proposed methods gave lower values relative to the theoretical ones indicating high accuracy and precision with no significant differences when compared to the official or reported methods. Therefore, these reagents can be safely used for quality control of Saquinavir Mesylate in their pure and in tablet dosage form.

Relative Standard Deviation values were low that indicates the reproducibility of the proposed methods. Recovery studies were close to 100% that

Table 1: Optical characteristics and precision data

	Method A	Method B
λ_{max} (nm)	435	486
Beer's law limits (mcg/mL)	2.0-25	5- 25
Ringbom conc. Range (mcg/mL)	3.5-23.5	6.5-22
Molar absorptivity (l/mol.cm)	3.85×10^3	2.12×10^3
Sand ell's sensitivity(micrograms/cm^2/0.001 absorbance unit)	0.298	0.376
Regression Equation* (Y)		
Slope (m)	0.008	0.217
Intercept (c)	0.146	0.044
Correlation Coefficient(r)	0.9999	0.9998
Precision (%Relative Standard Deviation)	0.417	0.952
Range of error		
Confidence level of 0.05	0.25	0.51
Confidence level of 0.01	0.68	0.89

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

Table 2: Assay of saquinavir mesylate in tablets

Sample No.	Labelled Amount (mg)	% Obtained± S.D* by proposed method		** % Recovery by the Proposed method	
		Method A.	Method B	Method A	Method B
1	500	100.1±0.45 t=0.35 F=1.72	99.9±0.17 t=1.2 F=2.72	99.8±0.33	100.7±0.54
2	500	99.5±0.22 t=0.58 F=1.92	100.5±0.56 t=0.85 F=2.2	100.2±0.39	99.6±0.78

Average ± standard deviation of six determinations, the t- and F-test values refer to comparision of the proposed method with the reference method. Theoretical values at 95% confidence limit , t=2.57 and F=5.05.
** Average of three determinations

indicates the accuracy and precision of the proposed methods.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sand ell's sensitivity are presented in Table 1.

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 1.

In conclusion, the proposed methods are simple, economical, sensitive, precise reliable and reproducible for the routine estimation of Saquinavir Mesylate in bulk as well as in tablet formulation.

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