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Development and validation of an ultraviolet spectrophotometric method for Saquinavir estimation in bulk and dosage form

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Abstract We developed and validated a simple, rapid, sensitive, precise, and reproducible specific UV spectrophotometric technique for determining Saquinavir in bulk medication and pharmaceutical dosage form. Methods: As per ICH recommendations, a simple double-beam UV spectrophotometric technique has been designed and validated with several criteria, such as linearity, precision, repeatability, the limit of detection (LOD), limit of quantification (LOQ), and accuracy. Results: UV-visible spectrophotometric approach, measurement of absorption at the maximum wavelength in 10 mL methanol, and volume made with water solvent system as reference Saquinavir was discovered at 239 nm. The medicine followed Beer's rule and showed a good correlation. Beer's law was followed for Saquinavir in the 1-5µg/ml concentration range, with a correlation coefficient of 0.999. Saquinavir's LOD and LOQ were determined to be 0.56 µg/ml and 2.78 µg/ml, respectively. The proposed method is simple, precise, accurate, and reproducible, and it may be utilized for routine analysis of Saquinavir in bulk and tablet dosage form.

Keywords: rapid method, accurate, beer's law

1. Introduction

The anti-retroviral medicine saquinavir (SQR) is structurally related to the related pharmaceuticals famciclovir and tenofovir (2S) -N-[(2S,3R) -4-[(3S) -3-(tert-butyl-carbamoyl)-decahydroisoquinolin-2-yl] -3-hydroxy-1 phenylbutan-2-yl] — 2-(quinolin-2-ylformamido) butanediamide (Figure 1)—the protease inhibitor saquinavir. Proteases are enzymes that hydrolyze, or break apart, protein molecules. HIV replication within a cell and the release of mature viral particles from an infected cell both need the presence of HIV protease. HIV-1 and HIV-2 proteases are blocked by saquinavir (Figgitt 2000; Winston 2006).

Bioanalytical method validation in protein-free human plasma for the antiretroviral drugs amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine, and efavirenz (Mu 2021) using extensive liquid chromatography; a novel RP-HPLC method for estimation of the protease inhibitor saquinavir (Mu 2021). Proust (2000) Nelfinavir, Indinavir, Ritonavir, Saquinavir, and Amprenavir in human plasma using a liquid chromatography-mass spectrometry technique (Proust 2000). RP-HPLC method for simultaneous quantifying indinavir, amprenavir, ritonavir, saquinavir, and nelfinavir in human plasma (Sarasa-Nacenta 2001).

Determination of Multiple HIV Drugs Using Reversed-Phase High-Performance Liquid Chromatography (HPLC)in Human Plasma (Droste 2003), Determination of Multiple HIV Drugs Using Reversed-Phase High-Performance Liquid Chromatography in Human Plasma (Notari 2006), and Determination of Multiple HIV Drugs Using Reversed-Phase High-Performance Liquid Chromatography in Human Plasma (Hoetelmans 1997) High-performance liquid chromatography for the simultaneous detection of anti-HIV medicines in human plasma (Notari 2006). It used ion-pair high-performance liquid chromatography with UV detection (Hoetelmans 1997) to quantify saquinavir in human plasma, saliva, and cerebrospinal fluid. Saquinavir, an HIV-1 protease inhibitor, was measured in the brain and testes of mice using high-performance liquid chromatography (Mudigonda 2006). Saquinavir concentration in Caco-2 cell monolayers using high-performance liquid chromatography-ultraviolet (HPLC-UV) (Ucpinar 2003). According to the literature, as mentioned earlier review, there is currently no method that has been described for the estimate of a drug that is simple, precise, reproducible, and accessible in terms of sample preparation. Therefore, a spectroscopic approach for the quantification of saquinavir in bulk and capsule dosage form using 20% methanol was devised in the present work. The established strategy is very targeted, accurate, and validated. In terms of cost, this

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strategy is efficient. The developed procedure was validated by ICH and USP standards (Savic 2008; USP 2003). On the validation data, the appropriate statistical tests were run (Bolton 1997).

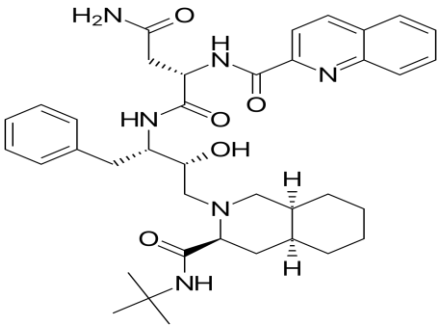

Figure 1 Structure of Saquinavir.

2. Materials and Methods

2.1. Materials and reagents

In the present investigation, Analytical method validation was performed using a Shimadzu UV/Visible twin beam spectrophotometer (Model 1700) and 1cm matched quartz cells. Saquinavir was provided as a gift sample by R. S. I. T. C. in Jalgaon. They used HPLC-grade methanol (Merck Specialities Pvt. Ltd., Shiv Sager Estate 'A' Worli, Mumbai) for the UV technique. The solvent employed in the investigation was a mixture of methanol and acidic water (0.05% OPA) made in double-distilled water. Saquinavir, 500 mg tablets, was purchased from a drugstore (Maximum 500- Cipla Pharmaceuticals Ltd).

2.2. Stock solution preparation

2.2.1. Stock Saquinavir solution

10 mg of Saquinavir (SQR) was dissolved in Methanol in a 10ml volumetric flask to make a 1000 µg/ml solution

2.2.2. Preparation of Stock Standard Solution

Carefully measure 10 mg of the Saquinavir working standard into a 10 ml volumetric flask and dilute it with Methanol until you get a concentration of 1000 µg/ml (stock solution). Once the standard has been sonicated for 15 minutes to dissolve, 0.1 ml of the stock solution should be transferred to the 10 ml volumetric flask - the result (Figure 2).

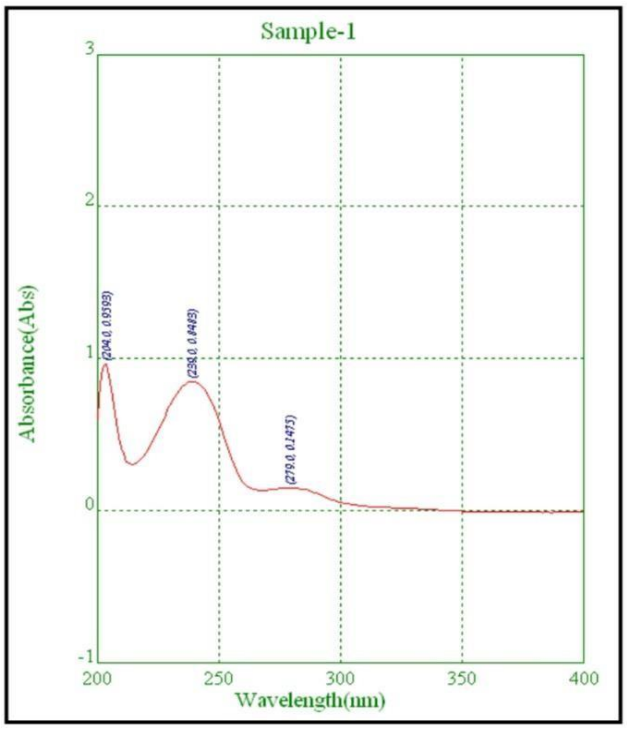

Figure 2 UV Spectrum of Saquinavir.

2.3. Procedure for calibration curve of Saquinavir

When the results from the calibration trials were analyzed using linear regression, a linear relationship was discovered between the peak regions and concentrations between 1 and 5 µg/mL of Saquinavir (Table 1). Saquinavir's concentration was best described by the linear equation y = 0.202x-0.195, where x represents the concentration and y represents the area of the peak obtained by UV spectrometry. The correlation coefficient was 0.999. Saquinavir's standard curve for dose-response is shown in (Figure 3).

2.4. Selection of detection wavelength

Saquinavir has its absorbance maximum (lambda max) at 239 nm when scanned against 10 ml of Methanol, and a volume made with a water solvent system is used as a standard.

Table 1 Linearity data for Saquinavir.

Conc µg/ml	Peak área (µV.sec) 1	2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
1	0.3938	0.3944	0.39	0.00	0.11
2	0.6026	0.6035	0.60	0.00	0.11
3	0.8059	0.8056	0.81	0.00	0.03
4	1.001	0.999	1.00	0.00	0.14
5	1.204	1.209	1.21	0.00	0.29
Equation			Y = 0.202x+0.0195		
R^2			0.999		

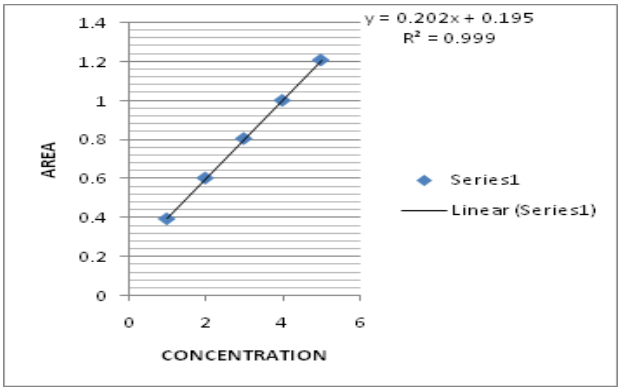

Figure 3 Calibration curve of Saquinavir.

2.5. Procedure for analysis of tablet formulation

Accurately weigh and transfer the sample amounting to 13.3mg Saquinavir into a 10 ml volumetric flask (20 tablets were used to get the average weight of 1330 mg). Sonicate it in roughly 10 ml of diluents to completely dissolve it, then add more diluents to bring the volume up to the required level. Incorporate thoroughly, then filter through a 0.45 µm membrane. As mentioned above, pipetanother 0.2 ml of the stock into a 10 ml volumetric flask, and dilute to the desired concentration (20 µg/ml) with the appropriate solvents. Saquinavir doses per tablet were determined by extrapolating the calibration curve's area value. Five separate iterations of the analysis technique were performed with the tablet iteration. Marketed formulation analysis included labeling as well. Evaluation: 99.99–101.00% successful results achieved (Table 2).

Table 2 Analysis of marketed formulation.

Drug	Label Claimed	Amt. Found	% Lable Claim	SD	% RSD
SQR	2	2.03	101.50	0.02	07
	2	2.04	101.50	0.01	07

2.6. Method validation

The linearity, precision, accuracy, limit of detection, and limit of quantification of the suggested methods have all been validated in compliance with ICHQ2 (R1) standards (Sudha 2012; Prasad 2012; Mante 2017; Ahmad 2022).

3. Results

3.1 Linearity and range

When the results from the calibration trials were analyzed using linear regression, a linear relationship was discovered between the peak regions and concentrations between 1 and 5 µg/mL of Saquinavir (Table 1). Saquinavir's concentration was best described by the linear equation y = 0.202x-0.195, where x represents the concentration and y represents the area of the peak obtained by UV spectrometry. The value of the correlation coefficient was a perfect 1.00. Saquinavir's standard curve for dose-response is shown in (Figure 3).

3.2 Accuracy

To ensure the efficacy of the new approach, recovery tests were conducted. Table 3 shows the results of adding standard medication at three different concentrations (80%, 100%, and 120%) to the already-analyzed tablet solution and calculating the recovery. Studying the recovery at 100%, 100%, and 120% concentrations confirmed the accuracy of the UV spectroscopic approach. The range of the percentage recovery was found to be 99-101%.

3.3 Statistical Analysis

Verification of the statistical recoveries is mentioned in Table 4. The results were analyzed using GraphPad Prism software, the quick calls option online. The Mean, Standard Deviation was calculated after taking at least three to six readings for UV Method. Relative Standard Deviation (RSD) and Correlation coefficient were also calculated.

Table 3 Result of recovery data for Saquinavir.

Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
	80%	2	1.6	3.62 ± 0.01	2.02 ± 0.07	101.03 ± 0.80
SQR	100%	2	2	1.012 ± 0.01	2.03 ± 0.01	101.5 ± 0.35
	120%	2	2.4	1.09 ± 0.01	2.43 ± 0.16	102.08 ± 0.29

*mean of each three reading for the UV method.

Table 4 Statistical validation of recovery studies Saquinavir.

Drug	Level (%)	Mean % Recovery	Standard Deviation*	% RSD
	80%	101.89	0.98	0.96
SQR	100%	97.53	1.27	1.30
	120%	101.58	0.21	0.20

*Denotes an average of three determinations for the UV method.

3.4 Precision

Saquinavir test method precision was investigated to characterize intra- and inter-day shifts. Analysis of three concentrations in three replication measurements within the linearity range of medications at three different times on the same day was used to calculate intraday precision (Table 6). The system was run generally for three days while interday precision was measured. The high precision% amount from 98% to 100% suggests the analytical method that concluded Table 5 from the research of intraday and interday precision on UV method for Saquinavir.

Table 5 Result of Intra day and Inter day Precision studies for Saquinavir.

Drug	Conc (µg/ml)	Intraday Precision		Interday Precision	
		Mean± SD	%Amt Found	Mean± SD	%Amt Found
	2	1.974 ± 0.01	99.77	2.015 ± 0.01	102.70
SQR	3	2.994 ±0.02	100.08	2.973 ±0.02	99.20
	4	3.926 ±0.01	99.89	4.0132 ±0.01	101.25

*Mean of each 3 reading for UV method.

3.5 System suitability parameters

Table 6 Repeatability studies on the UV method for Saquinavir.

Conc	Absorbance	Amt Found	% Amt Found
3	0.8055	3.02	100.67
3	0.8053	3.02	100.67
3	0.8056	3.02	100.73
3	0.8059	3.02	100.80
3	0.8053	3.02	100.67
	Mean	3.02	100.71
	SD	0.00	0.06
	%RSD	0.06	0.06

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

Under the specified experimental conditions, LOD is the minimal analyte concentration in a sample that can be detected. The limit of quantification (LOQ) refers to the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy using the methods and equipment available. Both 0.56 and 2.78 micrograms per milliliter were determined to be the LOD and LOQ, respectively, for saquinavir.

4. Discussion

The proposed approaches for the simultaneous estimate of saquinavir in tablet dosage forms were straightforward, precise, cost-effective, and quick (Shukla et al 2022). ICH Q2 (R1) standards confirmed the method to be reliable. At these particular wavelengths, the correlation coefficient (r2) for saquinavir obtained from standard calibration was 0.999. Recovery rates for both medicines came close to 100%, and the values of % RSD were well within the allowed range of 2%. Examining pharmaceutical formulations shows that the suggested method can simultaneously determine these components with minimal interference from other additives (Chavan et al 2022).

To summarize, saquinavir in formulations can be successfully estimated simultaneously using the abovementioned techniques (Ahmad 2022). UV-Vis spectroscopy can only be used to measure solutions, which is its primary limitation. It cannot be used to measure samples of solids or gases. Also, it is affordable and easily accessible. Further Studies can be done towards the development or refinement of saquinavir-based therapies. These specific methods and results presented can assist scientists across the globe in developing saquinavir-based therapies and their quality control (Gaikwad et al 2011).

5. Conclusion

Results showed that the new UV spectrophotometric approach was more exact, accurate, and repeatable than previous methods regarding linearity, precision, range, and robustness. In addition, it was observed that the procedures were straightforward and efficient. Therefore, all the methodologies presented are practical for use in quality assurance labs as part of their everyday procedures.

Abbreviations

UV stands for ultraviolet. HPLC stands for High-Performance Liquid Chromatography. HPTLC is for High-Performance Thin Layer Chromatography, and ICH stands for International Conference on Harmonization. UPLC stands for Ultra-Performance Liquid Chromatography. SQR stands for Saquinavir; RSD stands for Relative Standard Deviation; RT stands for Retention Time; and SD stands for Standard Deviation.

Ethical considerations

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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