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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF SAQUINAVIR IN PURE FORM AND PHARMACEUTICAL DOSAGE FORM

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Abstract

RP-HPLC method was developed for Saquinavir in bulk and pharmaceutical dosage form with a maximum absorbance found to be at 240nm and peak purity was excellent. The method was developed by using mobile phase ACN: Methanol (60:40% v/v) at a flow rate of 1ml/min using Symmetry ODS C18 (4.6 x 150mm, 5 m)column. The following method has been validated as per the ICH guidelines. The method has been validated for Accuracy, Precision, Linearity, System suitability, Specificity, Robustness. The method showed linearity in a range of 10, 20, 30, 40 and 50 µg / ml. The accuracy for 50%, 100% and 125% was found to be 100.42%. the retention time is found to be 3.155 min. It is found that the method of RP-HPLC with UV-detection system for the analysis of Saquinavir is straight forward and applied in qualitative and quantitative analysis. This method is simple, rapid, selective and inexpensive. The proposed method for estimation of selected drug Saquinavir was successfully applied in pharmaceutical formulation.

Keywords: RP-HPLC, Saquinavir formulation.

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INTRODUCTION

Saquinavir is (2*S*)-*N*-[(2*S*,3*R*)-4-[(3*S*,4*aS*,8*aS*)-3-(*tert*-butylcarbamoyl)-3,4,4*a*,5,6,7,8,8*a*-octahydro-1*H*-isoquinolin-2-yl] -3-hydroxy-1-phenylbutan-2-yl]-2-(quinoline-2-carbonylamino)butanediamide.

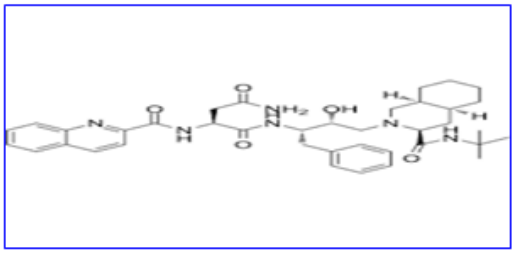

Fig.1.Chemical structure of Saquinavir

Saquinavir is usually used in combination with other medicines in the treatment of the infection caused by the human immunodeficiency virus (HIV). HIV is the virus that causes acquired immune deficiency syndrome (AIDS). Saquinavir will not keep you from spreading HIV to other people. People who receive this medicine may continue to have other problems usually related to AIDS or HIV disease. Saquinavir is a potent HIV protease inhibitor. It is used in combination with other antiviral drugs in the treatment of HIV in both adults and children. Saquinavir is a protease inhibitor: it inhibits HIV-1 and HIV-2 proteases. HIV protease is an aspartate protease which splits viral protein molecules into smaller fragments and it is vital to both the replication of the virus within the cell, and also to the release of mature viral particles from an infected cell. Side effects are Hives; difficulty breathing; swelling of your face, lips, tongue, or throat, easy bruising, unusual bleeding (nose, mouth, vagina, or rectum), purple or red pinpoint spots under your skin chest pain (especially when you breathe), dry cough, wheezing, feeling short of breath. Medical Uses are This drug is used with other HIV medications to help control HIV infection. It helps to decrease the amount of HIV in your body so your immune system can work better. This lowers your chance of getting HIV complications (such as new infections, cancer) and improves your quality of life. Saquinavir belongs to a class of drugs known as protease inhibitors.

EXPERIMENT

Equipment and Apparatus Used

HPLC WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector, pH meter Labindia, Digital ultra sonicator Labman, Weighing machine Sartorius, Volumetric flasks Borosil.

Selection of Mobile phase

A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drug. The mobile phase containing

- Acetonitrile: water (80:20% v/v)
- Methanol: Water (70:30)
- Water: ACN (25:75)
- Methanol: Acetonitrile (80:20)
- Methanol: Acetonitrile (30:70)
- ACN: Water (65:35)
- ACN: Methanol (60:40% v/v)

Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Methanol (60:40% v/v) respectively.

Preparation of mobile phase: Accurately measured 600 ml (60%) of HPLC Acetonitrile and 400 ml of Methanol (40%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45µ filter under vacuum filtration.

Selection of column: The method was performed with various C18 columns like Symmetry, Zodiac, Xterra. Symmetry ODS C18 (4.6 x 150mm, 5 [redacted] good peak shape and resolution at 1ml/min flow.

Preparation of standard solution: Accurately weigh and transfer 10 mg of Saquinavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of sample solution: Take average weight of the Powder and weight 10 mg equivalent weight of Saquinavir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3ml of the above Saquinavir stock solutions

into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula.

Quantitative Estimation

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Process Validation

The proposed High Performance liquid chromatographic process was validated as per the accordance of ICH guidelines with aspect to linearity, accuracy, precision, specificity and robustness.

Linearity

Accurately weigh and transfer 10 mg of Saquinavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance criteria

Correlation Coefficient should be not less than 0.9990.

Accuracy

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak response Calculate the Amount found and Amount added for Saquinavir and calculate the individual and mean recovery value.

Acceptance criteria

The mean % recovery of the Saquinavir at each spike level should be not less than 98.0 % and not more than 102.0%.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow conditions

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Acceptance criteria

The tailing factor of standard should be not more than 2.0 for Variation in flow.

The % RSD of Asymmetry and t_R of Saquinavir standard should be not more than 2.0% for variation in flow. Effect of Variation of mobile phase organic composition. The sample was analyzed by variation of mobile phase i.e. ACN: Methanol was taken in the ratio and 65:35, 55:45 instead of 60:40, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Acceptance criteria

Tailing Factor of Saquinavir standard should not be more than 2.0 for Variation in composition of mobile phase. The % RSD of Saquinavir standard should be not more than 2.0 for Variation in composition of mobile phase.

System Suitability

Accurately weigh and transfer 10 mg of Saquinavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3ml of the above Saquinavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Saquinavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock

solution) Further pipette 0.3ml of the above Saquinavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Take average weight of the Powder and weight 10 mg equivalent weight of Saquinavir sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3ml of the above Saquinavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol. Inject the three replicate injections of standard and sample solutions and calculate the assay.

Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

All individual assays of Saquinavir injection should be within 98% - 102% & Relative standard deviation of % Assay results should not be more than 2.0%.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Acceptance criteria

%RSD of 6 replicate preparations of assay should be not more than 2%.

RESULTS

Chromatographic Parameters

Column : Symmetry ODS C18 (4.6 x 150mm, 5 [redacted]

Column temperature : Ambient

Wavelength : 240 nm

Mobile phase ratio : ACN: Methanol

Flow rate : 1.0mL/min

Injection volume : 10 µl

Run time : 8 minutes

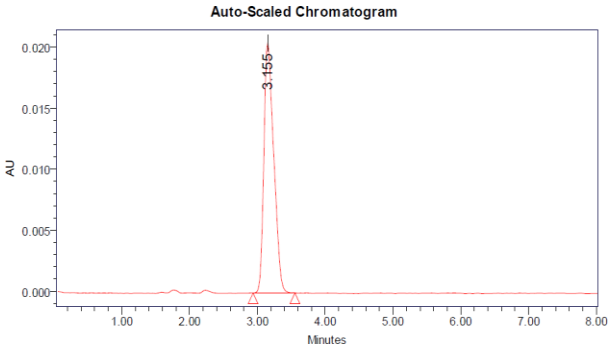

Figure. 1: Optimized chromatogram.

ASSAY

Table. 1: Estimation of Saquinavir in dosage

S.no	Name	RT	Area	Height	USP	USPPlate	Injecto
1	Saquinavir	3.170	224596	20469	1.35	6098	1
2	Saquinavir	3.174	224658	20489	1.34	6108	2
3	Saquinavir	3.170	224585	20458	1.35	6107	3

Validation Parameters Linearity

Table. 2: Standard Concentration to peak response for Saquinavir.

Concentration µg/ml	Average Peak Area
10	78683
20	146545
30	213584
40	279895
50	346568

Calibration curve of Saquinavir

Accuracy

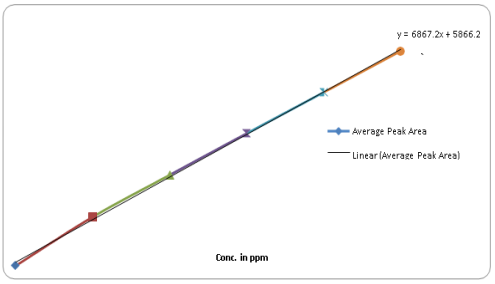

Table. 3: Accuracy results of Saquinavir.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109283.3	15	15.060	100.40 %	100.42 %
100%	212732	30	30.124	100.413 %	
150%	316263.3	45	45.201	100.446 %	

Robustness

Table. 4: Effect of variation in mobile phase and mobile phase composition.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

Specificity

Table. 5: Results of Assay (Standard) for Saquinavir.

Parameter Used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

System Suitability

Table. 6: System suitability parameters of Saquinavir.

System suitability parameter	Observed value	Acceptance criteria
USP Tailing	1.36	In between 0.5 to 2.0
USP Plate count	6125	Not less than 2000

Precision

a. **Repeatability**

Table. 7: Standard Chromatogram values for Repeatability of Saquinavir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Saquinavir	3.173	225487	20542	6253	1.35
2	Saquinavir	3.134	225484	20532	6098	1.36
3	Saquinavir	3.161	225364	20541	6254	1.35
4	Saquinavir	3.174	226513	20534	6235	1.36
5	Saquinavir	3.199	225487	20549	6199	1.36
6	Saquinavir	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std. Dev.			553.0524			
% RSD			0.244918			

b. **Intermediate Precision**

Table. 8: Standard Chromatogram values for Analyst 1 intermediate precision of Saquinavir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Saquinavir	3.165	226534	20653	6235	1.35
2	Saquinavir	3.163	226542	20598	6198	1.36
3	Saquinavi	3015	225989	20653	6254	1.36

	r	8				
4	Saquinavir	3.167	226512	20548	6281	1.35
5	Saquinavir	3.171	226531	20653	6199	1.36
6	Saquinavir	3.171	225898	20658	6253	1.35
Mean			226334.3			
Std. Dev.			304.2622			
% RSD			0.13443			

Table. 9: Standard Chromatogram values for Analyst 2 intermediate precision of Saquinavir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Saquinavir	3.173	225487	20542	6253	1.35
2	Saquinavir	3.134	225484	20532	6098	1.36
3	Saquinavir	3.161	225364	20541	6254	1.35
4	Saquinavir	3.174	226513	20534	6235	1.36
5	Saquinavir	3.199	225487	20549	6199	1.36
6	Saquinavir	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std. Dev.			553.0524			
% RSD			0.244918			

SUMMARY AND CONCLUSION

Although various methods have been reported for estimation of Saquinavir individually, but an attempt was made to develop an analytical method by RP-HPLC which is economically effective and can be used in industry for both qualitative and quantitative analysis. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Saquinavir was Very poorly Soluble in water, Very soluble in methanol, ethanol, and acetonitrile. Practically insoluble in soybean oil, mineral oil. ACN: Methanol (60:40 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP- HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Saquinavir in bulk drug and in Pharmaceutical dosage forms.

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