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Research Article

METHOD DEVELOPMENT AND VALIDATION OF LOPINAVIR IN TABLET DOSAGE FORM USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

SUNKARA NAMRATHA[1], VIJAYALAKSHMI A[2]*

[1]Department of Analysis, Bharat Group of Institutions, Ibrahimpatnam, Hyderabad, India. [2]School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India. Email: avijibaskaran@gmail.com

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ABSTRACT

Objective: Reversed-phase high-performance liquid chromatographic method (RP-HPLC) was developed for the assessment of lopinavir in the dosage form of tablet.

Methods: Chromatogram was run through using Kromosil C_{18} 4.5×150 mm using a mobile phase methanol: water of ratio 65:35% v/v with a rate of flow of 0.8 ml/min, measured by UV spectrometric detection at 265 nm. The method developed was validated in terms of precision, accuracy, linearity, and robustness parameters.

Results: Retention time of lopinavir established at 2.482 min and percentage R.S.D of lopinavir found to be 1.0% and 0.5%, respectively. The method shows that good linearity range of 30–150 µg correlation coefficient of lopinavir was 0.997. The limit of detection was 2.97 and limit of quantification was 9.92, respectively. The percent purity of lopinavir was 99.87%.

Conclusion: The suggested method (Rp-HPLC) for concurrent assay lopinavir was validated, which is appropriate method for the analysis of lopinavir quantitatively in tablet dosage forms and bulk.

Key words: Validation, Method development, Lopinavir, Reversed-phase high-performance liquid chromatography.

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INTRODUCTION

The selected drug lopinavir is a white to light (tan) powder, in water practically insoluble, but in methanol, it is freely soluble [1]. It is an anti-HIV, anti-infective, and antiretroviral agents [2]. Lopinavir inhibits the HIV viral protease enzyme. Detailed literature survey for the lopinavir determination in formulations and bulk drug revealed several methods that have been reported for the assay of it either alone or in combined dosage forms such as high-performance liquid chromatography (HPLC) [3-11] and UV spectrophotometric methods [12-17].

The development and validation of an analytical method is to ensure a specific, accurate, and precise method for a particular drug with an objective to enhance the conditions and parameters. From the literature survey that the reversed-phase HPLC (RP-HPLC) methods were not reported in the estimation of lopinavir. Thus, the present research paper describes the assessment of lopinavir by RP-HPLC method in tablet dosage form.

MATERIALS AND METHODS

Materials and instrumentation

Waters, separation module 2695, PDA detector Instrument with Kromosil C_{18} column 4.5×150 mm and HPLC - auto sampler - UV detector using Empower software version-2 and Lab India U.V double beam spectrometer of UV 3000+ model and U.V win software were used. HPLC grade water, methanol, acetonitrile, orthophosphoric acid, and $KH_2PO_4K_2HPO_4$ were procured from Merck Enterprises, India.

Lead of solutions and reagents

Standard solution preparation

About 10 mg of standard sample was weighed accurately, added to a volumetric flask of 10 ml capacity, and added 7 ml of diluents, dissolved completely by sonication and with stock solution the volume made up to the mark. To a volumetric flask of 10 ml capacity, 0.9 ml of stock solution was added and the solution was diluted to the mark using diluents.

Preparation of sample solution

To a 10 ml volumetric flask added accurately weighed 10 mg equivalent of lopinavir capsule powder, 1 ml of diluents was added, dissolved completely by sonication and with stock solution the volume made up to the mark. 1 ml of the prepared stock solution stated above was added to a volumetric flask of 10 ml capacity, and using diluents, the solution was diluted up to the mark.

Methodology

The chromatographic system was injected with about 20 µL of the blank solution, sample solution, and standard solution each, and lopinavir peak area was calculated.

Process validation

The proposed HPLC process validated in accordance of the ICH guidelines with aspect to accuracy, linearity, precision, specificity, limit of quantification (LOQ), robustness, and limit of detection (LOD) [10].

Linearity

The standard stock solution for both the drugs prepared individually by diluting to obtain the five standard solutions in the concentration of linearity range of 30–150 µg for lopinavir. About 20 µl volume was injected and run under above referred chromatographic conditions. From the values of peak area versus the concentration (µg/ml), linear regressions of lopinavir were executed. Linearity was checked with correlation coefficient and calibration curve.

Accuracy

The fidelity method was committed in solutions made of various concentrations of lopinavir, that is, 50%, 100%, and 150%, the quantity of sample lopinavir is kept constant and amount of pure drug was varied in terms such as 5 mg, 10 mg, and 15 mg in case of lopinavir, i.e., 50%, 100%, and 150%, respectively.

Repeatability, LOD, and LOQ [4,7]

Assay preparation on same day during intraday precision (repeatability) was resolved by replicate analysis (n=3); intermediate precision was checked on 3 consecutive days for replicate analysis of the given solutions. Assay precisions were conveyed as relative standard deviation.

The following equation indicates the LOD from the calibration curve [3,7].

$$LOD = 3.3 \times \frac{D}{S}$$

Hence, y-intercept gives standard deviation and S is slope of line.

The LOQ was calculated as follows [3,7]:

$$LOQ = 10 \times \frac{D}{S}$$

LOD and LOQ concentrations of test solution were injected 6 times in the chromatograph and R.S.D. percent was measured from the peak area of replicate injections.

Robustness

The precision study was performed for five injections of lopinavir. Each standard injection was injected in to chromatographic system.

The area of each Standard injection was used for calculation of % RSD.

As the part of the method validation robustness, the impact of flow rate change deliberately and the mobile phase composition change with the method was evaluated [4].

a. Lopinavir standard solution 90 ppm was prepared and by varying flow rates (0.8–1.2 ml/min) along with flow rate method analyzed [6].
b. Lopinavir standard solution 90 ppm was prepared and analyzed by varying mobile phase composition from 75% to 55% along with the actual mobile phase.

System suitability

To 10 ml volumetric flask added 10 mg of lopinavir standard sample by weighing accurately and sonicated by adding 7 ml of diluent, using stock solution, the volume makes up to the mark. To volumetric flask of 10 ml capacity added 0.9 ml lopinavir from the above stock solution, and using diluent, the solutions were diluted up to the mark.

RESULTS AND DISCUSSION

The suggested method originated as mere, specific, precise, and accurate for the routine estimation of drugs simultaneously. Several mobile phases with dissimilar solvents in varied proportions tried to resolve the drug peaks with fair peak asymmetry, resolution, and number of theoretical plates. Finally, a mobile phase consisting of 65:35% v/v methanol: water was selected for analysis, which showed good resolution of lopinavir peak with a retention time of 2.482 min, and neither of the impurities was interfering in its assay. System suitability trails were accomplished to specify resolution, column (Fig. 1) efficacy, peak asymmetry, and tailing factor. For the proposed method, all the system suitability criteria were within the limits specified and findings are stated in Table 1.

Linearity

The correlation coefficient was calculated by plotting a graph with concentration in the X-axis and Y-axis peak area. It was found to be 0.999. Correlation coefficients, slopes, and y-intercepts of regression lines for the two substances were evaluated and results are incorporated in Table 2.

Accuracy

The percent recovery of lopinavir ranged out of 98.0–102.0%, respectively. Therefore, no interference from the additives commonly present in the tablets and developed technique constitute to be precise as the percent relative standard deviations for repeatability and intermediate precision is <2 as per proposed ICH guidelines. The value of 1.0% for lopinavir indicates good repeatability of the method. The recovery studies data are stated in Table 3.

LOD and LOQ

LOD for lopinavir was 2.97 and LOQ for lopinavir is 9.92 g/mL and 10.02 g/mL, respectively.

Precision

The %RSD of Lopinavir was found to be 0.15 (Table 4). %RSD values of peak area found to be less than 2.0. Hence the optimized method was found to be precise as per ICH guidelines Q2 (R1).

Robustness

The robustness considerate varied chromatographic conditions, the retention time and peak asymmetry of the two drug peaks were not significantly influenced by the low standard deviation values (below 2)for each criterion. Thus, the developed LC method was robust in the findings of lopinavir in combined tablet dosage formulations Table 5.

System suitability

Accurate amount of 10 mg lopinavir standard was weighed and the standard drug was transferred to volumetric flask of 10 ml capacity, sonicated after adding diluent of about 7 ml to dissolve the components and stock solution added to make volume up to the mark. Pipetted 0.9 ml of the prepared stock solution stated above to standard flask of 10 ml capacity and diluted using diluent up to the mark.

Assay

The proposed technique was applied in terms of assay for commercial formulation by injecting the blank solution, standard solution, and sample solution of 20 µL each into the chromatographic system. The percentage of lopinavir was calculated from the peak areas. The percent purity of lopinavir was found to be 99.87%. The assay findings and label

Table 1: System suitability parameters for lopinavir

System suitability parameter	Lopinavir
Retention time (min) (mean±S.D., n=5)	2.428
Tailing factor (peak asymmetry)	0.87
Theoretical plates	4024

Table 2: Calibration data of lopinavir

Name	Rt	Area
Lopinavir	2.428	1,608,152
Lopinavir	2.422	2,592,905
Lopinavir	2.430	3,778,327
Lopinavir	2.426	5,170,038
Lopinavir	2.433	6,249,400
Coefficient of correlation (R2)		0.997

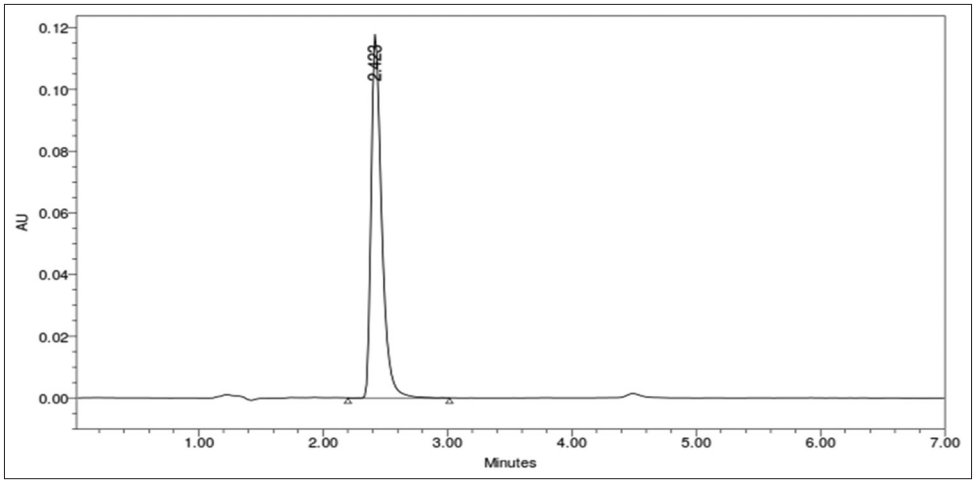

Fig. 1: Typical high-performance liquid chromatography chromatogram of lopinavir

Table 3: Accuracy of lopinavir

% concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% recovery	Mean recovery
50	1,048,287	5	5.14	100.2	100.4%
100	1,378,200	10	10.01	98.8	
150	1,715,480	15	15.2	96.5	

Table 4: Precision of lopinavir

Name: LOPINAVIR			
S. No	**Name**	**Rt**	**Area**
1	LOPINAVIR	2.423	693,078
2	LOPINAVIR	2.424	693,338
3	LOPINAVIR	2.424	695,080
4	LOPINAVIR	2.424	694,843
5	LOPINAVIR	2.423	695,336
Mean			694,335
SD			1047.5
% R.S.D.			0.15

Table 5: Robustness studies for lopinavir

S. No	Flow rate (ml/min)	System suitability results	
		USP plate count	USP tailing
1	0.8	4352	1.1
2	1	4024	1.2
3	1.2	3730	1.2

Table 6: System suitability parameters for lopinavir

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP plate count	USP tailing
1	10% less	4331	1.20
2	*Actual	4024	0.87
3	10% more	3693	1.26

claim of the substance had good agreement between them. This implies the disposition of drug in tablet dosage form was steady without significant variation. The result of estimation is presented in Table 6.

Result analysis of tablet formulation

Liquid chromatographic method was developed in this study determine the lopinavir amount in combined tablet dosage forms. The total chromatographic analysis time per sample was 5 min with respect to the lopinavir retention time established at 2.482 min and percentage R.S.D. of lopinavir found to be 1.0% and 0.5%, respectively.

CONCLUSION

For concurrent assay of lopinavir, method suggested (RP-HPLC) was validated and it was an appropriate method for routine analysis of lopinavir quantitatively in dosage forms and. The proposed technique is accurate, rapid, and sensitive. It makes the use of little quantities of solvents and changes the set of conditions requires in short intervals. It does not suffer from any hindrance due to common additives present in pharmaceutical dosage forms and can be readily accepted for quality control analysis.

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