

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF LOPINAVIR IN BULK AND IN PHARMACEUTICAL DOSAGE FORM

***Namratha Sunkara and A.Vijayalakshmi**
Vels University, Pallavaram, Chennai, India
*Author for Correspondence

ABSTRACT

A new, simple, precise and accurate UV- spectrophotometric method was developed and validated for the determination of lopinavir in pure and tablet dosage form. Lopinavir exhibited maximum absorption at 203nm in methanol and obeyed linearity in the concentration range of 10-50 µg/ml. The proposed method was statistically validated. From the results obtained for precision, it was found that % RSD is less than 2% it indicates that the proposed method has good reproducibility. The linearity coefficient of lopinavir was found to be 0.999 and accuracy it was found that percentage recovery values of pure drug from the analyzed formulation was between 98.6-102.2 which indicates that the method is accurate and commonly used excipients and additives present in the formulation was not interfering in the proposed method. The UV-spectrophotometric method was extensively validated for linearity, accuracy, precision, LOD and LOQ. All these analytical validation parameters were observed and the % RSD was determined which indicates the usefulness of method for determination of lopinavir in bulk drug and tablet formulation.

Keywords: Lopinavir, Validation, UV-Spectrophotometric, Accuracy

INTRODUCTION

Lopinavir (The Indian Pharmacopoeia Commission Ghaziabad, 2007) is chemically known as (2S)-N-[(2S,4S,5S)-5-[2-(2,6dimethylphenoxy) acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide and its empirical formula is C₃₇H₄₈N₄O₅ with a molecular weight of 628.80. Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-polyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles. The chemical structure was shown in figure 1. Literature review revealed that very few methods was reported for determining of lopinavir in bulk and pharmaceutical dosage form by UV-spectrophotometric methods (The Indian Pharmacopoeia Commission Ghaziabad, 2007; Seshachalam *et al.*, 2007; Ponnilaravasan *et al.*, 2010; Suneetha *et al.*, 2011; World Health Organization, 2014). Hence, in the present work an attempt was made to develop simple, precise and accurate analytical method for estimation of lopinavir in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Materials

Triple distilled water, Methanol, which is of AR grade, was used for the analysis. A reference standard sample of lopinavir is procured from Mylan labs, Hyderabad. Commercial Lopinavir tablets (Norvir 100mg by Abbott Laboratories Pharmaceuticals) was used in the analysis.

Instrumentation

Spectral and absorbance measured were made on an UV spectrophotometer – UV 1800- shimadzu. By using 1cm quartz cells. Shimadzu – type BL -220 H electronic balances was used for weighing the samples.

Method

Preparation of stock solution

Standard stock solution was prepared by dissolving 10 mg of drug in 100 ml of Methanol to get concentration of 1mg/ml (100 µg/ml) solutions.

Preparation of Working Standard Solutions and construction of standard graph

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The prepared stock solution was further diluted with Methanol to get working standard solutions of 100 µg/ml of Lopinavir. To construct Beer's law plot for pure drug, different concentrations (10-50µg/ml) was taken and diluted to 10 ml with Methanol. The absorbance was measured maximum at 203nm against Methanol as blank. The result was shown in table. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Figure 2 the drug has obeyed Beer's law in the concentration range of 10-50µg/ml.

Estimation of lopinavir in commercial formulation

Tablets were procured from local market (Norvir 100mg by Abbott Laboratories Pharmaceuticals) and average weight was determined. The powder equivalent to 100mg of lopinavir was weighed accurately and dissolved in 100ml of methanol and filtered using whatmann filter paper # 42. The filtrate was appropriately diluted with methanol to give standard stock solution of 100µg/ml. Further dilutions were made using methanol to give 10, 20, 30, 40, 50 µg/ml. Absorbance was measured at 203nm against standard solution.

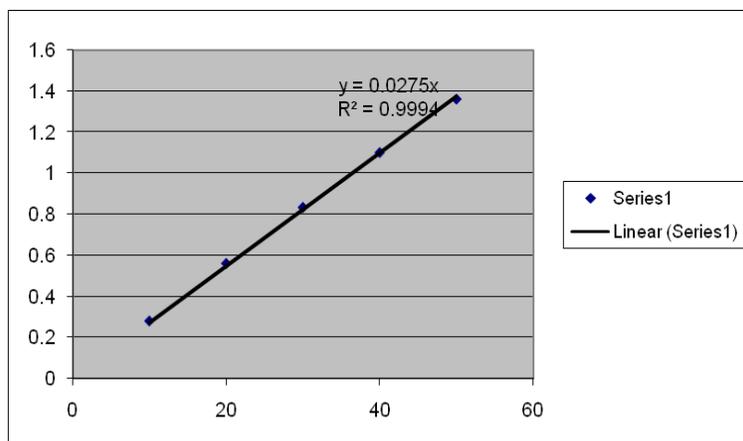
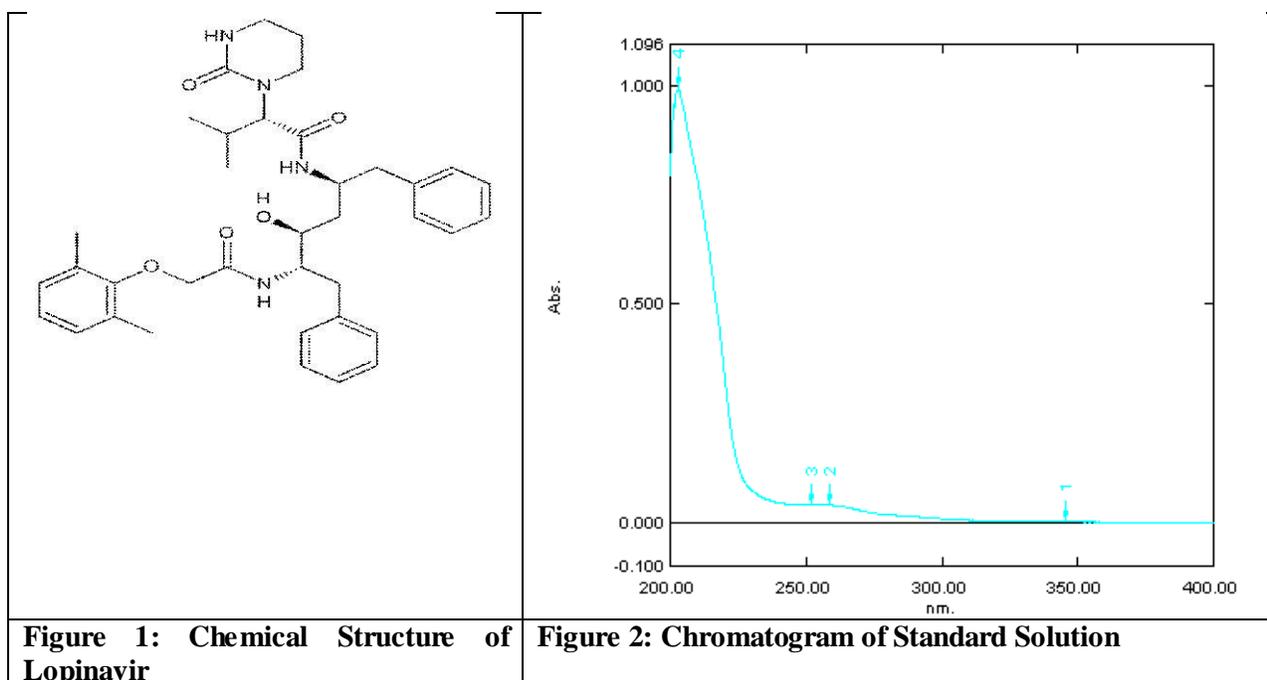


Figure 3: Linearity Graph of Lopinavir

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RESULTS AND DISCUSSION

Optimization

Scanning and Determination of Maximum Wavelength (λ_{max}):

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different solutions of the drug (10 - 50 $\mu\text{g/ml}$) in methanol was scanned using spectrophotometer within the wavelength region of 200 – 400nm against methanol as blank. Lopinavir shows λ_{max} at 203nm. The resulting spectra were shown in figure 2 and the absorption curve showed characteristic absorption maxima at 203nm for lopinavir.

Precision:

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by proposed method. From the absorbance Mean, Standard Deviation, % R.S.D, % Range of errors (at 0.05 and 0.01 confidence limit) was calculated. The readings were shown in table 4.

Accuracy: An Accuracy study was carried out by standard addition method. Pure Lopinavir was added at different levels i.e. 80%, 100% and 120% to drug sample present in tablet dosage form (100 mg Lopinavir in each coated tablet).

Limit of Detection: It was calculated from the values of calibration curve and it was found to be 0.231 $\mu\text{g/ml}$.

Limit of Quantization: It was calculated from the values of calibration curve and it was found to be 0.377 $\mu\text{g/ml}$.

Table 1: Linear Regression Data for Calibration Curves

Drug	Lopinavir
Concentration range, $\mu\text{g/ml}$	10-50
Slope, m	0.04577
Intercept	0.7321
Correlation coefficient	0.9994
% RSD	0.9

Table 2: Results of Assay and Recovery Studies

S. No	Amount Taken ($\mu\text{g/ml}$)	Amount of Drug Found ($\mu\text{g/ml}$)	% Recovery
1	10	10.22	102.2
2	20	19.72	98.6
3	30	29.23	97.4
4	40	39.75	98.5
5	50	50.88	101.19

Table 3: Statistical Evaluation of Recovery Studies

Level Recovery	% Mean	$\pm\text{S.D.}^*$	%RSD*
80	98.6	0.786	0.795
100	99.3	0.8798	0.887
120	99.8	0.5886	0.592

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Table 4

Concentration in µg/ml	Absorbance at 203 nm	Statistical Analysis Lopinavir
30	0.831	Mean : 0.831
30	0.831	
30	0.822	
30	0.832	S.D : 0.00358
30	0.831	
30	0.821	
30	0.831	%RSD : 0.432
30	0.831	
30	0.823	
30	0.831	

Conclusion

It is clear from the present study that the prescribed method of analysis is simple, accurate, specific and precise in operation and can be employed for routine batch analysis of lopinavir in tablets.

ACKNOWLEDGEMENT

The authors acknowledge Mylan Ltd, Hyd., for providing authentic gift sample of lopinavir.

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