Original Article

A first-derivative spectrophotometric method for the estimation of Lopinavir in tablets

Abstract

The present study aimed at the development and validation of a first-derivative ultraviolet (UV) spectrophotometric method for the estimation of Lopinavir in formulation as well as in bulk drug. The reported methods of analysis include chromatographic methods such as high-performance liquid chromatography using a UV detector. Chromatographic methods are tedious, time consuming and are not suitable for routine analysis. However, UV-based methods are fast, simple and economical. Thus, these methods are preferred for routine analysis. The absorption scan of Lopinavir in the UV region does not exhibit any sharp peak. However, the first-derivative spectrum showed an absorption minima at 220 nm, which was selected as the analytical wavelength. Thus, a first-derivative method was developed and validated for the routine analysis of Lopinavir. The method was found to be simple, sensitive and selective, as evidenced by non-interference from the excipients and ritonavir present in the tablet formulation. The linearity was observed in the range of 5 to 35 µg/ml. The method was found to have a high degree of accuracy and precision, both inter- and intra-day. The limit of detection and limit of quantitation were found to be 0.844 and 2.558 µg/ml, respectively.

Access this article online

Website
http://www.cysonline.org

DOI
10.4103/4444-4443.76450

Quick Response Code

Key words:
Derivative spectrophotometry, Lopinavir, validation

Introduction

Lopinavir (2S)-N-[(2S,4S,5S)-5-[[2-(2,6-dimethylphenoxy) acetyl]amino]-4hydroxy-1,6-di(phenyl)hexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide is an antiretroviral drug used in the treatment of acquired immunodeficiency syndrome.[1] The molecular formula of Lopinavir is $C_{37}H_{48}N_4O_5$.[1] The structure of the drug is shown in Figure 1. It inhibits the human immunodeficiency viral proteinase enzyme, which prevents cleavage of the gag–pol polyprotein, resulting in non-infectious, immature viral particles. It is freely soluble in most of the organic solvents like acetonitrile, methanol and chloroform, but it is practically insoluble in water.[1] Lopinavir is official in USP 2010 and IP 2007. The official method of assay in both IP 2007 and USP 2010 is high-performance liquid chromatography (HPLC).[2-3] The other reported methods of analysis include chromatographic methods such as HPLC using different detectors and mobile phase compositions.[4-7] Chromatographic methods are tedious and time consuming. Thus far, only one UV spectrophotometry-based method is reported for the estimation of Lopinavir.[8] However, the reported ultraviolet (UV) method is found to have less sensitivity as evidenced by the high value of the limit of quantitation (LOQ) (10 µg/ml). Lopinavir does not exhibit a sharp peak when scanned in the UV region. The first-derivative spectrum shows a sharp absorption minimum at 220 nm. Thus, in the present investigation, a first-derivative spectrophotometric method was developed and validated.

Materials and Methods

Materials

Lopinavir was obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad, India. Acetonitrile AR was purchased from Fisher Scientific, Mumbai, India. Lopinavir tablets (Lopimune, Cipla Ltd., India) were purchased from a retail pharmacy in Vadodara.

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How to cite this article: Thakkar HP, Patel KH. A first-derivative spectrophotometric method for the estimation of Lopinavir in tablets. Chron Young Sci 2010, 1:22-25

Equipment/instruments

Analytical balance (Precisa 205A SCS, Switzerland), UV-Visible Spectrophotometer (Shimadzu UV-1700, Japan) with inbuilt UV probe 2.10 software and Bath sonicator (Model 120W, Vibronics Pvt. Ltd., Mumbai, India).

Methods

Preparation of stock solution

The stock solution of concentration 1000 µg/ml was prepared by dissolving 50 mg of Lopinavir in 50 ml of acetonitrile.

Determination of analytical wavelength

Accurately measured 0.3 ml of the stock solution of Lopinavir was transferred to a 10 ml volumetric flask. Then, it was diluted up to the mark with acetonitrile to obtain a solution of strength 30 µg/ml. The resulting solution was scanned in the UV region of 400–200 nm using acetonitrile as the blank. The zero-order spectrum was then converted to the first-derivative spectrum using UV probe software (scaling factor-5, delta λ-2). The wavelength at which there was an absorption minimum was selected as the analytical wavelength.

Linearity

Appropriate aliquots of the stock solution were transferred into separate 10 ml volumetric flasks and diluted with acetonitrile to get concentrations ranging from 5 to 35 µg/ml. The first-derivative spectrum was recorded for each solution and the absorbance difference (dA/dλ) was calculated by the inbuilt software of the instrument. The above procedure was repeated six times and the mean of the determinations was calculated. The mean absorption difference (dA/dλ) was plotted against the concentration of the solutions to get a calibration curve.

Stability

Stability of the solutions of Lopinavir used for preparing the calibration curves was ascertained by observing the changes in the absorbance difference (dA/dλ) at 220 nm over a period of 24 h, at room temperature.

Precision

The precision of the method for the estimation of the drug in the presence of various excipients used in the tablet was investigated by performing an assay of the commercially available tablets (Lopimune, Cipla Limited) containing 200 mg of Lopinavir per tablet. Twenty tablets were powdered and the powder blend was mixed properly. An accurately weighed amount of the powder containing 200 mg of Lopinavir was transferred to a 100 ml volumetric flask. Lopinavir was extracted by ultrasonication from this blend using acetonitrile. The extract was filtered using whatman filter paper No. 1 and, after suitable dilution, was scanned between 400 and 200 nm using a UV-Visible spectrophotometer. The first-derivative spectrum was obtained and the absorbance difference (dA/dλ) at 220 nm was determined. The amount of Lopinavir present was calculated using the regression equation. The assay of the tablet was performed in six replicates, and the mean and the relative standard deviation values were calculated. Data of intra-day precison are shown in Table 1.

Interday precision was evaluated by performing an assay of the tablets by the above-mentioned procedure on three different days. Data of interday precision are shown in Table 2.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by the standard addition method at three different levels, 80%, 100% and 120%. The powder blend of the pre-analyzed tablets containing 200 mg of Lopinavir was taken in a 100 ml volumetric flask and acetonitrile was added up to mark. A measured volume of this solution was mixed with standard Lopinavir solution and diluted suitably to get total Lopinavir concentrations of

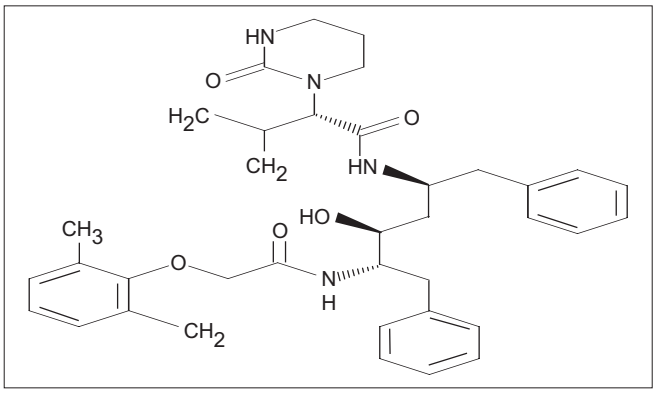

Figure 1: Structure of Lopinavir

Table 1: Data for intra-day precision

Labeled amount	Amount estimated/ tablet (mg)	Percentage of labeled amount
200 mg	204.18	102.09
	204.6	102.30
	203.58	101.79
	200.93	100.46
	201.86	100.93
	200.9	100.45

Table 2: Results of precision of the proposed method

Labeled amount	Day	*Mean amount estimated (mg±SD)	*Percentage of the labeled amount (%±SD)
200 mg	Day 1	202.53±2.07 mg	101.33±0.97
	Day 2	201.63±1.13 mg	100.45±0.64
	Day 3	201.87±0.89 mg	100.67±0.67

*indicate (n=3)

27, 30 and 33 μg/ml, respectively. The absorbance difference of the solutions was measured at 220 nm. The study was performed in three replicates and the mean was found at each concentration level.

Selectivity

The selectivity of the methods for the estimation of Lopinavir in the presence of excipients and Ritonavir was investigated by performing the assay of commercially available Lopinavir tablets.

Limit of detection and LOQ

The values of Limit of detection (LOD) and LOQ were found by the formula (9) CL or $Q_L=K^*S_B/S$, where K is a constant, the value of which is 3.3 for LOD and 10 for LOQ, S_B is the standard deviation of the response and S is the slope of the concentration versus absorbance difference (dA/dλ) graph.

Results and Discussion

The zero-order absorption spectrum of Lopinavir does not show any sharp peak in the UV region and, hence, selection of analytical wavelength is difficult. The first-order derivative of the spectrum was recorded and is shown in Figure 2. It can be seen from the first-derivative spectrum that a sharp absorption minima is observed at 220 nm. Thus, the further studies were performed using 220 nm as the analytical wavelength. The solvent selection was carried out on the basis of the solubility of Lopinavir. Various solvents suitable for analysis were tried, and it was found that Lopinavir exhibited maximum solubility in acetonitrile and in methanol. However, the analytical wavelength of 220 nm does not allow the use of methanol as there is significant noise at around 220 nm when methanol is used. Hence, acetonitrile was used as the solvent for the analysis. Table 3 shows the mean absorbance difference (dA/dλ) at the various concentrations along with the standard deviation values. It can be seen that Lopinavir obeys Beer's law in the concentration range of 5–35 μg/ml, and there is a linear relationship between absorbance difference (dA/dλ) and concentration, with a high correlation coefficient value of 0.999. The solutions prepared for the study of the linearity were stored for 24 h at room temperature and the readings were taken again. There was no significant change in the readings obtained after 24 h, indicating the stability of the solutions over the period of 24 h.

The precision of the method was evaluated using the standard deviation of the results and the coefficient of variation between the six values of the Lopinavir content in the tablets found by performing the assay of the powder blend in six replicates. The values of relative standard deviation for interday and intra-day precision were found to be 0.815 and 0.236, respectively. These low values of the relative standard deviation indicate that the method is precise in estimating the content of Lopinavir from the tablet. The precision of the method for estimating Lopinavir in the active pharmaceutical ingredient is also supported by the very low standard deviation values of the absorbance difference (dA/dλ) of the solutions prepared for determination of linearity. The results summarized in Table 4 show that the amount added and amount estimated by the proposed method are very close. The accuracy of the method to estimate the correct amount of the drug added

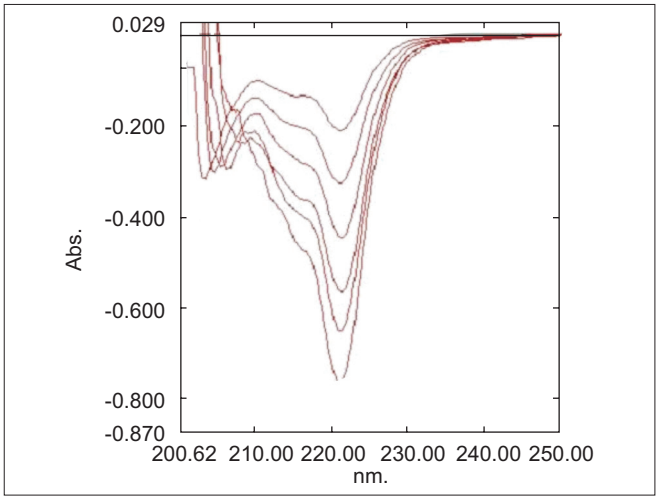

Figure 2: Graphical spectrum of Lopinavir at10–30 μg/ml in the first-derivative ultraviolet spectrum

Table 3: Mean absorption difference values along with standard deviation values at different concentrations of Lopinavir

Concentration (μg/ml)	Absorbance difference (dA/dλ)±SD
5	−0.114±0.007
10	−0.211±0.006
15	−0.325±0.009
20	−0.428±0.011
25	−0.54±0.004
30	−0.644±0.005
35	−0.762±0.008

Intercept (a)=0.00; Slope (b)=-0.021; Correlation coefficient=0.999; *Mean of six values; *n*=6

Table 4: Results of accuracy studies of the proposed methods

Level	Sr. no.	Concentration used (μg/ml)	Absorbance difference (dA/dλ)	Concentration found (μg/ml)	% recovery
80%	1	27	−0.573	27.11	98.79
	2	27	−0.591	27.95	101.89
	3	27	−0.569	26.93	98.10
100%	1	30	−0.650	30.69	99.08
	2	30	−0.663	31.30	101.06
	3	30	−0.651	30.74	99.23
120%	1	33	−0.701	33.06	99.43
	2	33	−0.718	33.86	101.84
	3	33	−0.719	33.90	101.98

was ascertained by using the "t"-test at each level. The computed "t" values at 80%, 100% and 120% were found to be 1.0498, 3.258 and 2.193, respectively. These values are lower than the tabulated "t" value of 4.30 ($P \leq 0.05$), indicating that no significant difference exists between the added and the estimated quantities. Thus, the method is accurate in estimating the Lopinavir content. This is further indicated by the low values of the relative mean error.

The first-derivative spectrum obtained from the standard solution overlapped with that of the spectrum obtained from the test solution prepared from Lopinavir tablets. The identical spectra obtained in both the solutions indicated that there is no analytical interference from ritonavir as well as the other excipients used in the tablet. The excipients do not interfere with the quantification of Lopinavir by the first-derivative spectroscopy, and it can be considered selective and specific in the estimation of Lopinavir. The sensitivity of the method can be evaluated by its LOD and LOQ. LOD is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. LOD represents the concentration of the analyte that would yield a signal to noise ratio of 3. The LOQ is defined as the lowest concentration of the analyte that can be accurately estimated. LOQ represents the concentration of analyte that would yield a signal to noise ratio of 10. The LOD and LOQ values for the proposed methods were found to be 0.844 and 2.558 μg/ml. These low values indicate that the method is sensitive enough for routine estimation. The LOQ value of the reported zero-order spectrophotometric method is 10 μg/ml, which indicates the lower sensitivity of the method compared with the first-derivative method. This is also supported by the fact that the concentrations used in the zero-order method for estimation are also very high compared with the concentrations used in this first-derivative method. Thus, the sensitivity of the first-derivative method is superior to that of the zero-order spectroscopic method.

Conclusion

The developed first-derivative spectrophotometric method is simple, sensitive and specific for the detection of Lopinavir in bulk and tablet formulations. It could precisely and accurately quantify Lopinavir in the presence of ritonavir and other tablet excipients. The high sensitivity of the method was evidenced by the low values of LOD and LOQ. Beer's law was found to be obeyed from 5 to 35 μg/ml. Thus, the proposed method is suitable for routine estimation of Lopinavir in bulk and pharmaceutical formulations.

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Source of Support: Nil, **Conflict of Interest:** None declared