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Research Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR DETERMINATION OF LOPINAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new, simple, precise and accurate RP-HPLC method was developed and validated for the determination of lopinavir in pure and tablet dosage form. The separation was carried out using phenomenex C_{18} (250 x 4.6 mm, 5 µm particle size) column, with a mobile phase consisting of acetonitrile and phosphate buffer (7.8) in the ratio of 85:15 v/v. The flow rate was set at 1.0 ml/min and detection was monitored at 215 nm. The retention time of lopinavir is 4.4 min respectively. The linearity coefficient of lopinavir was found to be 0.9999 and percentage recoveries for lopinavir is 99.85.The linearity was found in the concentration range of 150-350 µg/ml for lopinavir respectively. The liquid chromatography method was extensively validated for linearity, accuracy, precision, and robustness. 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, RP-HPLC, Acetonitrile and Buffer (pH 7.8).

INTRODUCTION

Lopinavir[1] is chemically known as (2*S*)-*N*-[(2*S*,4*S*,5*S*)-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 $C_{37}H_{48}N_4O_5$ with a molecular weight of 628.80. Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-polpolyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles. The chemical structure was shown in fig1. Literature review revealed that very few methods was reported for determining of lopinavir in bulk and pharmaceutical dosage form by HPLC[2-7] and spectrophotometric methods[8-10]. 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.

EXPERIMENTAL

Materials

Triple distilled water of HPLC grade, Methanol of HPLC grade, 0.2 M potassium dihydrogen ortho phosphate (KH_2PO_4) and 0.2 M sodium hydroxide (NaOH), which are of AR grade were used for the analysis. Reference standard samples of lopinavir is procured from Hetro labs,Hyderabad. Commercial Lopinavir tablets (Lopimune, Cipla Ltd., India) were used in the analysis.

METHOD

Gradient RP-HPLC-SHIMADZU LC 20 AD (prominence) and the Column specifications is C_{18} column (2), 250×4.6mm, 5µ particle size,

Injector-Rheodyne, UV-Visible Spectrophotometer- Perkin Elmer (lambda 25).

Preparation of mobile phase
Buffer preparation pH (7.8)
To 125 ml of 0.2 M potassium dihydrogen orthophosphate in 500ml volumetric flask add 111 ml of 0.2 M sodium hydroxide and the volume was made up to 500ml with water. The pH was adjusted to 7.8 with 0.2 M sodium hydroxide. Acetronitrile and phosphate buffer were filtered through 0.45µ membrane filter and sonicated before use.

Preparation of stock and standard drug solutions of lopinavir
Stock solutions of Lopinavir is prepared by dissolving 25mg of each drug taken in a separate 25 ml volumetric flasks in methanol and volume is made to 25ml with methanol, sonicated for about 15 min. From the individual stock solutions, working standard solutions were prepared in a concentration range of 150-350µg/ml.

Estimation of the drugs from tablet dosage forms
Ten tablets of LOPIMUNE, containing lopinavir (200mg) was weighed and finely powdered. A quantity of the powder equivalent to 200mg of lopinavir was weighed, transferred in to 100 ml volumetric flask and dissolved in the mobile phase by sonication for about 15 min. This solution was filtered through 0.45µ filter paper. From the filtrate different aliquots were taken in separate 10ml volumetric flasks. The contents of the flask were made up to the volume with methanol and mixed well. Then these samples were injected and peaks were recorded.

Calibration curve
Separate standard calibration curves were prepared for each drug. Different volumes of stock solutions were accurately transferred to a 10ml volumetric flask to 150-350µg/ml concentration range for Lopinavir respectively. Six replicate solutions in the above range were prepared for each concentration. The calibration curve was constructed by plotting the analyte peak area against concentration.

RESULTS AND DISCUSSIONS
Method optimization
The suitable parameters were chosen after several trails with buffers of different pH values and various compositions of acetonitrile and buffer. However the final concentration was adjusted to achieve good resolution. The trails revealed that with the decrease in acetonitrile concentration, the peak obtained was broad and showed severe tailing. The peak obtained with a composition of acetonitrile and Buffer 85:15v/v was proved to be most suitable of all the combinations since the peaks obtained were better defined and resolved and free from tailing. To determine the effect of flow rate, the method was performed at different flow rates 0.5ml/min, 0.7ml/min, 1.1ml/min and 1.2ml/min. The optimum flow rate 1ml/min was chosen finally. The retention time obtained for lopinavir is at 4.45 min and the chromatogram was shown in figure 2. Validation was carried out and validation summary was tabulated in table 3.

Linearity and calibration
Varying quantities of the standard stock solution was diluted with the mobile phase to give concentration of 80%, 100% and 120% of the Lopinavir. The injections were made at an interval of 15 min and the peak area was determined. A calibration curve was determined by plotting the peak areas obtained against concentrations. There exists a linear relationship showing concentrations ranging for Lopinavir from 150µg/ml to 350µg/ml. From the data obtained, correlation coefficient for the Lopinavir was found to be 0.999. Linear regression data for calibration curves was shown in the table 1. The resulting linearity plot was shown in the figure 3.
The number of theoretical plates was 7618 and tailing factor was 0.8. The retention time was 4.45 min for the developed RP-HPLC method. The number of the theoretical plates was high indicating the efficient performance of the column.

Precision
Repeatability expresses the precision under the same operating conditions. The R.S.D in the present experimentation was found to be 0.955%. The low R.S.D indicates that the method is precise and accurate.

Recovery studies
Determination of accuracy by direct comparison to reference standard is a preferred technique. Recovery studies were performed by spiking the blank matrix of the sample at different levels (80%, 100%, and 120%) of the known level in the sample. Average recovery of the analyte was found to be in the range of 99.4-100.8 at different levels of spiking.
The developed RP-HPLC method utilizes ACN& buffer (pH-7.8) in the ratio of (85:15) as a mobile phase and phenomenex C_{18} column as a stationary phase. The method precision

and system precision were performed and found to be within the limits. The recovery study reveals the accuracy and precision of the method employed for the present studies and the results are shown in table 2.

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.

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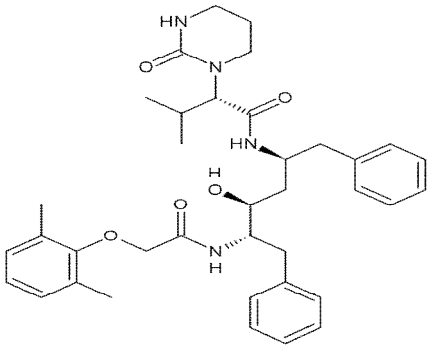

Fig. 1: Chemical structure of lopinavir

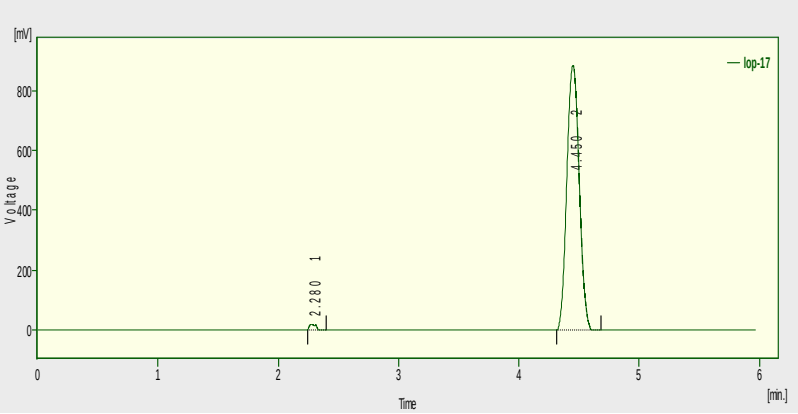

Fig. 2: Chromatogram of standard solution

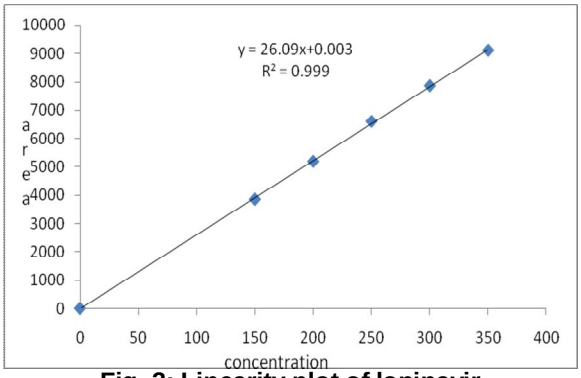

Fig. 3: Linearity plot of lopinavir

Table 1: Linear regression data for calibration curves

Drug	lopinavir
Concentration range, µg/ml	150-350
Slope, m	26.09
intercept	0.003
Correlation coefficient	0.9999
%RSD	0.9

Table 2: Results of assay and recovery studies

Sample	Amount claim (mg / tablet)	Amount found (mg/tablet)	% Recovery*
1.	5	5.06	100.8
2.	6	5.95	99.4

*Average of two different concentration levels.

Table 3: Validation summary

Validation parameters	Results
Theoretical plates(N)	7618
Linearity range, mcg/ml	150-350
Tailing factor	0.8
R_t (min)	4.45
LOD, µg/ml	11.92
LOQ, µg/ml	36.121

REFERENCES

1. The Indian pharmacopoeia commission Ghaziabad. Indian Pharmacopoeia,2007, 2, 685.
2. Seshachalam U, Haribabu B and Chandrasekhar KB. A novel validated LC method for quantitation of lopinavir in bulk drug and pharmaceutical formulation in the presence of its potential impurities and degradation products. Biomedical Chromatography. 2007;21(7):716–723.
3. Ponnilavarasan I, Rajasekaran A, Dharuman JG, Kalaiyarasi D and Senthilkumar M. RP – HPLC method for simultaneous estimation of antiretroviral drugs lopinavir and ritonavir in tablet dosage form, Digest Journal of Nanomaterials and Biostructures, 2010; 5(3):771-778.
4. Suneetha A, Kathirvel S and Ramachandrika G. A validated RP HPLC method for simultaneous estimation of lopinavir and ritonavir in combined dosage form, International journal of pharmacy and pharmaceutical sciences. 2011;3(1):49-51.
5. Phechkrajang CM, Thin EE, Sratthaphut L, Nacapricha D and Wilairat P. Quantitative Determination of Lopinavir and Ritonavir in Syrup Preparation by Liquid Chromatography, Journal of Pharmaceutical Science. 2009;36(4):1-12.

6. Temghare GA, Shetye SS and Joshi SS, Rapid and Sensitive Method for Quantitative Determination of Lopinavir and Ritonavir in Human Plasma by Liquid Chromatography-Tandem Mass Specrtometry, E-Journal of Chemistry. 2009;6(1):223-230.
7. Myasein F, Kim E, Zhang J, Wu H and El- Shourbagy TA. Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC–MS/MS, Anal Chim Acta. 2009;651(1):112-116.
8. Thakkar HP and Patel KH. A first-derivative spectrophotometric method for the estimation of Lopinavir in tablets, Chron Young Sci. 2010;1(3):22-25.
9. Vaishali N and Kishore B. Simultaneous estimation of ritonavir and lopinavir by vierodt's UV spectrophotometric method in combined tablet dosage form, International Journal of Pharmaceutical Science. 2010;2(2):533-536.
10. Vaishali P, Nagulwar, Kishore P and Bhusari. Simultaneous Estimation of Ritonavir and Lopinavir by Absorption ratio (Q-analysis) UV Spectrophotometric Method in Combined Tablet Dosage Form. Der Pharmacia Lettre. 2010;2(1):196-200.