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Simple and sensitive analytical method development and validation of lopinavir bulk drug by RP-HPLC

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ABSTRACT

A simple, selective, accurate reverse phase - high Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the analysis of Lopinavir in bulk drug. Chromatographic separation achieved isocratically on a C18 column (Use Symmetry C18, 150 X 4.6mm, 5µ) utilizing a mobile phase of Acetonitrile: Methanol: Phosphate buffer (50:30:20v/v/v, pH 3) at a flow rate of 1.0ml/m with UV detection at 210nm. The retention time was 7.2667. The method is accurate (97.78-100.56%), precise (the % relative standard deviations of intra and inter-day were 0.172 and 0.199 respectively) and linear within range 1-150µg/ml (R^2=0.999) concentration and was successfully used in monitoring left over drug. The limit of detection and limit of quantification for Lopinavir drug was found to be 0.01µg/ml and 0.03µg/ml.

Key words: Lopinavir, RP-HPLC, method development, and Validation.

INTRODUCTION

Lopinavir is designated chemically as (S)-N-[(1S, 3S, 4S)-4-[[(2, 6-dimethylphenoxy) acetyl] amino]-3-hydroxy-5- phenyl-1-(phenyl methyl) pentyl] tetra hydro-(1-methylethyl)-2-oxo-1-(2H) Pyrimidineacetamide and is a prescribing drug for HIV protease inhibitors with the combination of other HIV drugs, belongs to protease inhibitors [1]. (Fig. 1).

Lopinavir (LPV) is a potent HIV protease inhibitor (PI) and a key ingredient of Highly Active Anti-Retroviral Therapy (HAART) [2]. LPV was developed by Abbott Laboratories to improve pharmacokinetics and to reduce HIV resistance of the company's earlier protease inhibitor, Ritonavir (RTV) [3]. LPV has low oral bioavailability when administered alone because of poor solubility, high first pass metabolism [4] and P-gp efflux [5]. RTV is co-administered with LPV orally in HAART in order to improve the bioavailability of LPV. RTV increases bioavailability of LPV due to its inhibitory effects on gut and liver Cytochrome (CYP) P450 enzymes and permeability glycoprotein (P-gp) efflux system [6].

Several research groups have been working on the development of novel delivery systems containing LPV alone in the effective treatment of HIV/AIDS. Such delivery systems will avoid heavy pill burden of LPV and RTV co-formulation, and improve patient compliance and adherence to therapy which are very vital for treatment against HIV/AIDS. In line with this, researchers have tried to improve solubility and bioavailability of LPV using micro particulate and nanocarrier systems [7, 8]. Agrawal and co-workers have tried prodrug approach for LPV [9]. Outcome of improving bioavailability of LPV using novel drug delivery systems containing LPV alone have been positive in preclinical studies, conducted on rats and mice [10]. Such research endeavours need a simple, rapid and cost-effective bioanalytical method to quantify the LPV concentration in rat plasma.

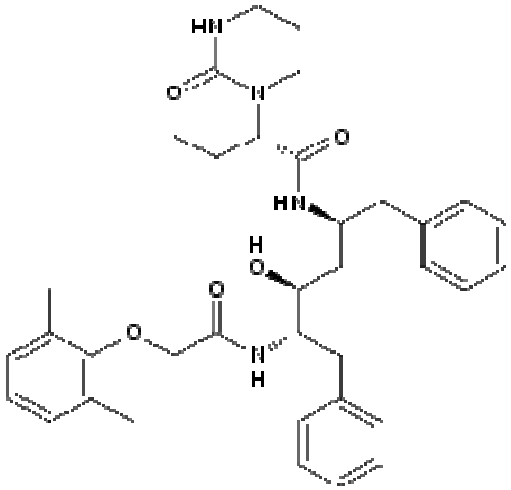

Fig. 1: Structure of Lopinavir

Analytical methods for PIs like amprenavir, indinavir, nelfinavir, ritonavir and saquinavir have been reported in human plasma and/or other biological matrices [11–21]. Simultaneous methods for estimation of LPV in combination with various PIs in human plasma, mainly by liquid chromatography tandem mass spectrometry (LC-MS), have been reported [22–29]. High-Performance Liquid Chromatographic (HPLC) methods with UV detection have also been explored successfully for simultaneous determination of LPV with other PIs using isocratic as well as gradient elution techniques [30, 31]. A method for estimation of LPV alone in human plasma matrix using HPLC system has been validated [32].

MATERIALS AND METHODS

Apparatus:

The analysis was performed by using the analytical balance Shimadzu Libror, Digital ph meter LABINDIA-PHNA, the HPLC used is of Younglin with UV detector. Column used in HPLC is Symmetry C18, 150 X 4.6mm, 5µ (isocratic). The mobile phase consists of A, B & C with mixture of Acetonitrile, Methanol and the Buffer (pH-3) at different proportions A, B, & C which are degassed in a sonicator for about 10minutes the injection volume is 20mL and the ultra violet detection was at 210nm.

Reagents and solutions:
Pure sample of Lopinavir and other reagents such as Acetonitrile, Methanol, Potassium dihydrogen Phosphate and water used were of HPLC and milli-q grade. All other chemicals like glacial acetic acid used were of AR grade. Optimized chromatographic conditions are listed in table.1.

Stock solution preparation:
Accurately weigh about 10mg of Lopinavir reference standard and transfer it into a 10ml volumetric flask. Add 5ml of methanol and kept in an ultrasonic bath until it dissolved completely. Make up to the mark with the methanol and mix. This yielded solution of 1000µg/ml concentration.

Standard solution preparation:
Spiked accurately about 0.5ml of Lopinavir stock solution and transfer it into a 10ml volumetric flask. Make up to the mark with the mobile phase and mix. This yielded solution of 50µg/ml concentration.

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution Limit of detection and Limit of quantification.

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

Accuracy: Accuracy for the assay of Lopinavir tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Lopinavir standard is added at different levels (80%, 100%, and 120%).

Linearity & Range: The Linearity of detector response is established by plotting a graph to concentration versus area of Lopinavir standard and determining the correlation coefficient. A series of solution of Lopinavir standard solution in the concentration ranging from about 5µg/ml to 150µg/ml level of the target concentration (100µg/ml of Lopinavir) were prepared and injected into the HPLC system.

RESULTS AND DISCUSSION

Table. 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Younglin with UV detector)
Column	Symmetry C18, 150 X 4.6mm, 5µ is suitable
Mobile Phase*	Acetonitrile: Methanol: Buffer (50:30:20v/v/v)
Flow rate	1.0ml/min
Detection	UV at 210nm
Injection volume	10µl
Column Temperature	Ambient
Runtime	10minutes

Lopinavir standard having concentration 50µg/ml was scanned in UV- region between 200- 400 nm. λ_{max} of Lopinavir was found to be at 210nm. The Lopinavir peak in the sample was identified by comparing with the Lopinavir standard and the Retention time was found to be around 7.2667minutes. The estimation Lopinavir was carried out by RP-HPLC using Mobile phase having a composition 500 volumes of Acetonitrile, 300 volumes of Methanol and 200

volumes of buffer (0.02M KH_2PO_4). The ratio pH was adjusted to be 3. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Symmetry C_{18}, (150 X 4.6mm, 5µ particle size). Flow rate of Mobile phase was 0.1ml/min.

System suitability parameters such as %RSD for six replicate injections were found to be 0.029%, theoretical plates – 7320.3, and tailing factor – 1.04. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show Method Repeatability 0.199% which shows that the method is precise. The validation of developed method shows that the drug stability is well within the limits. System suitability parameters are listed in table.2.

Table. 2: System suitability parameters

Parameter	Lopinavir
Calibration range (µg/ml)	1-150
Theoretical plates	7320.3
Tailing factor	1.04
Correlation Coefficient(r^2)	0.999
% Recovery	97.78% - 100.56%
System Suitability %RSD	0.029%
Method Repeatability %RSD	0.199%

The linearity of the detector response was found to be linear from 1 to 150µg/ml of target concentration for Lopinavir standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) = 0.999, which shows that the method is capable of producing good response in UV-detector.

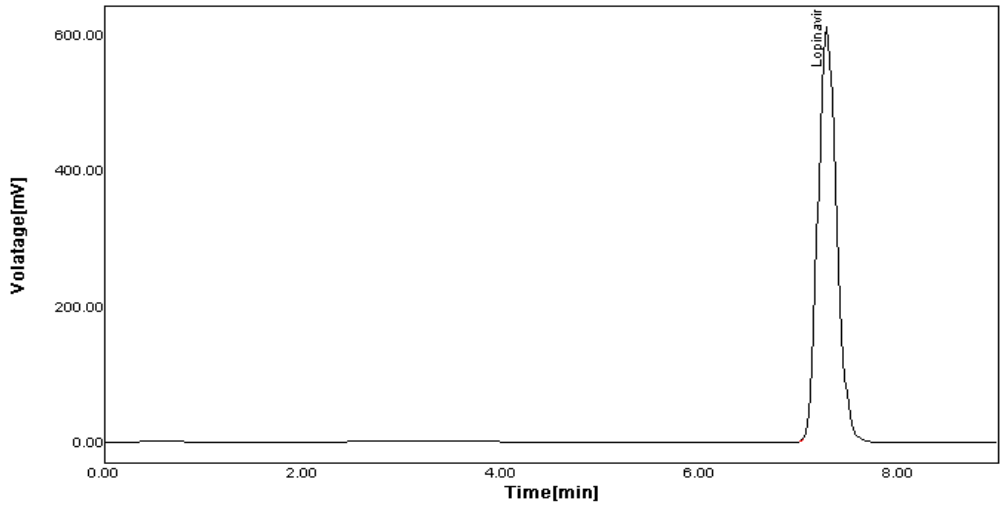

Fig. 2: Chromatogram of standard for Lopinavir

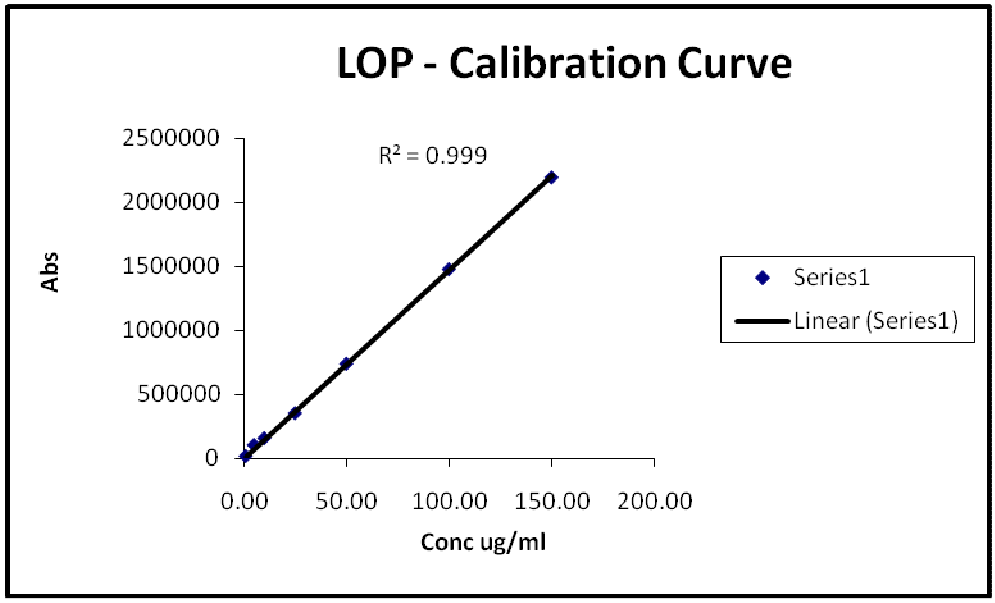

Fig. 3: linearity of Lopinavir

CONCLUSION

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Lopinavir is done by reverse phase HPLC. The mobile phase consists of 500 volumes of Acetonitrile, 300 volumes of Methanol and 200 volumes of buffer (0.02M KH_2PO_4). The ratio pH was adjusted to be 3. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The detection is carried out using UV detector set at 210nm. The solutions are chromatographer at the constant flow rate of 1.0ml/min. The Retention time for Lopinavir was around 7.2667minutes. Linearity range for Lopinavir is 1 to 150µg /ml. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 97.78-100.56% for Lopinavir. The results obtained on the validation parameter met the requirements. It inferred that the method was found to be simple, specific, precise, and linear, proportional i.e. it follows Lambert- Beer's law. The method was found to have a suitable application in routine laboratory analysis with a high degree of accuracy and precision.

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