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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF LOPINAVIR IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Lopinavir in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade Methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Lopinavir in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade Methanol, soluble in Isopropanol and practically insoluble in Water. So, the analytical grade Methanol is used as a diluent in method. The melting point of Lopinavir was found to be 257-258˚C (uncorrected). It showed absorption maxima 265 nm in analytical grade Methanol. On the basis of absorption spectrum, the working concentration was set on 10µg/ml (PPM). The linearity was observed between 6-14 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.7, 101 and 99.2% for three levels respectively. The % RSD for precision was found to be 0.62%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Lopinavir in tablet dosage form. The method could be considered for the determination of Lopinavir in quality control laboratories.

KEYWORDS: Lopinavir, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

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.[1] Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-polyprotein and, therefore, improper viral assembly results. Lopinavir (LPV) is an HIV (human immunodeficiency virus) protease inhibitor (PI) coadministered with a low dose of ritonavir (RTV) under the brand name Kaletra (LPV/r) as part of antiretroviral treatment (ART) in people affected by HIV.[2, 3] The combination was approved by the U.S. Food and Drug Administration (FDA) two decades ago. In the latest WHO guidelines (2019), LPV/r is still recommended as the preferred PI therapy for second-line ART regimen, alternative first-line ART regimen in children and in special circumstances in neonates.[4]

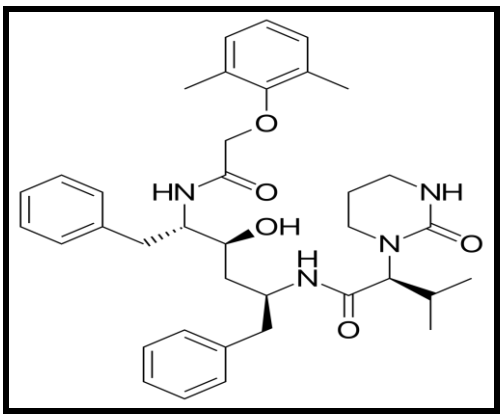

Fig. 1: Chemical Structure of Lopinavir.

From literature review it's found that lot of work was done on simultaneous estimation[5], Q- Method[6] and first order derivative[7] method for Lopinavir in combination with other drugs. Also, some HPLC[8, 9, 10, 11] methods, Impurity determination[12] found for Lopinavir and other drugs. Some blood plasma[13] method is also found for Lopinavir. But very few methods were reported on estimation of Lopinavir in tablet dosage form for UV method This indicates that so far, no UV method exists for the estimation and determination of Lopinavir in tablet dosage forms.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

- **Materials**

Lopinavir was obtained as a gift sample. Lopinavir tablets were procured from local pharmacy. Methanol used was of analytical grade. Glass double distilled analytical grade Methanol was used throughout the experiment. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (15 PPM)[14, 15, 16]

100 mg weighed amount of Lopinavir was dissolved into 100 ml of volumetric flask with analytical grade Methanol. Pipette out 1.5 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with analytical grade Methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

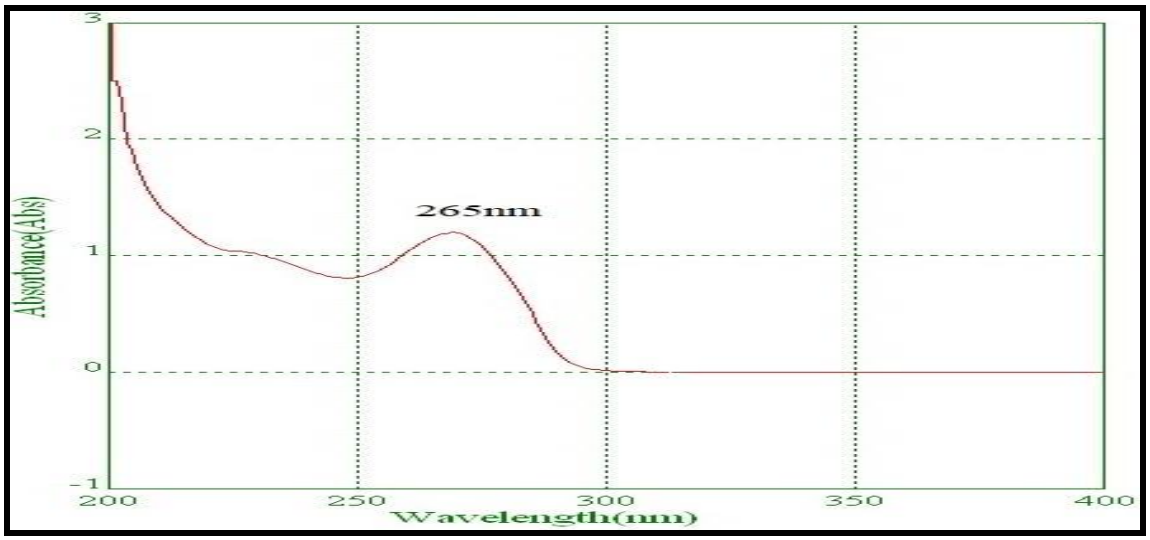

Fig. 2: Calibration Curve.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 100 mg of Lopinavir in 100 ml of analytical grade Methanol to get concentration of 1000 µg/ml (PPM).

Preparation of Standard solution

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with analytical grade Methanol to get concentration of 10 µg/ml (PPM).

C. Preparation of Working concentration

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D. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade Methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

E. Procedure for sample preparations[17, 18, 19, 20]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 100 mg of Lopinavir was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml analytical grade Methanol, the

solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade Methanol. Filtered the solution through whatmann filter paper. Pipette out 1 ml of the above solution and diluted up to 100 ml with analytical grade Methanol. The absorbance was measured at 265 nm. The absorbance was recorded.

Table 1: Absorbance of Dosage Form.

Emletra® Emcure Pharmaceutical Limited (100 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.6329
3	Sample	0.6308

Table 2: Dosage Form Specifications.

Type	Brand Name	M.D.	E.D.	Batch No.	Average weight (g)	Assay (%)
1	Emletra®(200mg) Emcure Pharma	02/2023	07/2025	HDF 2514	0.3827	99.67

F. Method of validation[21, 22, 23]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 6 - 14 PPM at 265nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	6 PPM	0.3967
2	8 PPM	0.5298
3	10 PPM	0.6395
4	12 PPM	0.7492
5	14 PPM	0.874
Correlation coefficient		**0.9988 ~ 0.999**

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade Methanol and measuring the absorbance at 265nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.81	100.92
100	1	1.02	101.86
120	1.2	1.18	98.55

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.6306
2	Sample Solution 2	0.6377
3	Sample Solution 3	0.6318
4	Sample Solution 4	0.6345
5	Sample Solution 5	0.6309
Mean		**0.6331**
SD		**0.0030**
% RSD		**0.4732**

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.6310	0.6313	99.74	0.0335
		0.6315			
2	Analyst 2	0.6307	0.6310	99.69	
		0.6312			

RESULTS

1. Solubility of Lopinavir

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Methanol, Ethanol	Freely Soluble
2	Isopropanol	soluble
3	Water	Practically insoluble

2. Melting point of Lopinavir

The melting point of Lopinavir was found to be 257-258˚C (uncorrected).

3. Results for linearity for assay method of Lopinavir

The linearity of method was determined at concentration level ranging from 6 to 14 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9988 ~ 0.999.**

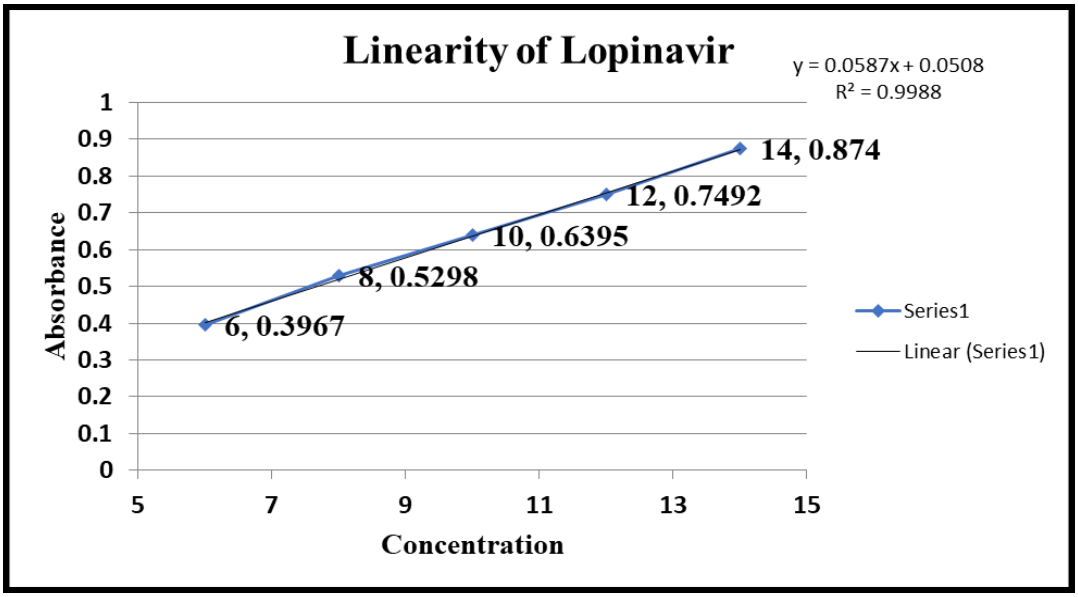

Fig. 3: Lopinavir Standard Curve.

4. Results for accuracy for assay method of Lopinavir

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Lopinavir

The % RSD for different sample of precision was found to be 0.4732 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Lopinavir

The %RSD for different sample of ruggedness was found to be 0.0335 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Lopinavir in tablet form has been developed. From the spectrum of Lopinavir, it was found that the maximum absorbance was 265 nm in analytical grade Methanol. A good linear relationship was observed in the concentration range of 6-14 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Lopinavir in solid dosage forms. Hence, the method could be considered for the determination of Lopinavir in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. LPV - Lopinavir
6. RTV – Ritonavir

7. PI - Protease Inhibitor
8. FDA - U.S. Food and Drug Administration
9. DNA - Deoxyribonucleic acid
10. HIV - Human Immunodeficiency Virus
11. ICH - International Council for Harmonization
12. RSD - Relative Standard Deviation
13. SD - Standard Deviation
14. Qty - Quantity
15. C - Celsius
16. M.D. - Manufacturing Date
17. E.D. - Expiry Date

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