International Journal of Engineering Science Invention (IJESI)
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org || PP. 08-12

SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF LOPINAVIR USING CHROMOGENIC REAGENTS

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Abstract: *Simple, accurate and reproducible UV-Visible spectrophotometric methods were established for the assay of LOP based on the oxidative coupling and condensation reactions. Condensation and coupling of the LOP with Ninhydrin-Ascorbic acid is proposed in method A. Method B includes condensation of LOP with Vanilin-H_2SO_4. The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (A-B) are given. Regression analysis using the method of least squares was made to evaluate the slope(b), intercept(a) and correlation coefficient (r) and standard error of estimation (Se) for each system.Determination of LOP in bulk form and in pharmaceutical formulations were also incorporated*

Keywords *: Estimation, Lopinavir, Condensation , Antiviral,*

I. Introduction

Lopinavir is a protease inhibitor. Lopinavir inhibits HIV protease, causing the enzyme incapable of processing the polyprotein precursor. This leads to the production of non infectious and immature HIV particles. The structural features, category; certain characteristics, therapeutic importance and commercially available formulations of LOP are compiled in tables 1 and 2 respectively.

A very few physico – chemical methods appeared in the literature for the assay of LOP in biological fluids and pharmaceutical formulations (less). The methods so far reported include UV and visible spectrophotometric methods [1-3], chromatography[4-6], HPLC[7] in biofluids, pharmacological [8] and clinical aspects, applied radiation and isotopes, polarographic [9-11] methods. The analytically useful functional groups in LOP have not been fully exploited for designing suitable, visible spectrophotometric methods and so still offer a scope to develop more visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing two methods i.e., [**VN (M_1); NIN-AA (M_2)**]. All these methods have been extended to pharmaceutical formulations as well.

TABLE 1: Sturctural Features Of Lopinavir

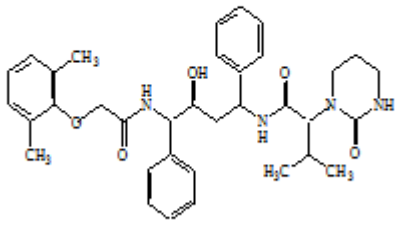
Generic Name	Chemical Name	Structure
Lopinavir	1(2H)-pyrimidine acetamide, N-[1S,3S,4S)-4-[(2,6-dimethyl-phenoxy) acetyl] -3-hydroxy-5-phenyl-1 (phenyl methyl) phenyl] tetra- hydro-α-(1-methyl ethyl)-2-oxo (α S).	

TABLE – 2: Physico Chemical Characteristic And Therapeutic Importance Of Lopinavir

Category	Characteristics	Mode of action and therapeutic use
Antiviral	**Molecular formula**: C_{37} H_{48} N_4 O_5 **Formula Weight** : 628.80 g/moles **Appearance:** White to light tar powder **Solubility**: Freely soluble in methanol and ethanol soluble in isopropanol; practically insoluble in water.	Lopinavir is a protease inhibitor. Lopinavir inhibits HIV protease, causing the enzyme incapable of processing the polyprotein precursor. This leads to the production of non infectious and immature HIV particles.

II. Experimental

2.1 Instruments used:

An Elico, UV – Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

2.2 Preparation of standard drug solutions:

An 1 mg/ml solution was prepared by dissolving 100 mg of pure LOP in 10ml of MeOH and this stock solution was diluted step wise with distilled water to get the working standard solutions of concentration of 200 $\mu g.mL^{-1}$ [M_1 and M_2]

2.3 Proposed procedures:

After systematic and detailed study of the various parameters involved, the following procedures [Methods **VN (M_1); NIN-AA (M_2)**] were recommended for the assay of **LOP** in bulk samples and pharmaceutical formulations.

2.4.1 For Bulk samples

2.4.1.1 Method - M_1

To each of 25ml calibrated tubes, aliquots (0.5-2.5 ml, 20$\mu g.ml^{-1}$) of standard **LOP** solution, 2.0mLof vanillin and 3mL of conc.sulphuric acid were added successively and the total volume in each flask was brought to 20mL by the addition of methanol and placed in heating water bath (maintained at 50^0C) for 15min. Then the flasks were colored and made up to the mark with methanol and the absorbance's were measured at 530nm against a reagent blank prepared in a similar way. The con of drug in a sample was computed from Beer-Lambert plot **(Fig. 3)**.

2.4.1.2 Method – M_2

Aliquot of standard **LOP** solution (0.5ml 2.5ml; 400$\mu g.ml^{-1}$) was transferred into a series of calibrated tubes containing 4.0mLof buffer (pH 5.0), 1.0mL ninhydrin (5.605×10^{-5}M) solution and 0.5mL of ascorbic acid (5.678×10^{-3}M) solution. The volume in each tube was adjusted to 8.0mL with distilled water and was kept in boiling water bath. After 15min tubes were removed and chilled in ice water. The solution in each tube was made up to 10.0mL with distilled water. The absorbance's were measured at 535nm after 10min against a reagent blank prepared similarly.The amount of **LOP** was calculated from its calibration graph **(Fig 4).**

2.4.2 Pharmaceutical formulations:

An accurately weighed portion of tablet content equivalent to about 100mg of **LOP** was transferred into a 100mL volumetric flask. Added about 80mL of warm isopropyl alcohol and shaken well for about 20min. The contents were diluted with isopropyl alcohol up to the mark and mixed thoroughly. The solution was filtered. The filtrate was evaporated to dryness. The residue was used for the preparation of formulation solutions for different methods as given under standard solutions preparations. These solutions were analyzed as under procedures described fro bulk solutions.

III. Results And Discussions

3.1 Spectral Characteristics:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of LOP were taken and colors were developed separately by

following the above procedures. The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in **Fig. 1** and **2,** The absorption curves of the colored species in each method show characteristics absorption maxima where as the blank in each method has low or no absorption in this region.

3.2 Optimum conditions fixation in procedures:

3.2.1 Method - M_1 [Vanillin]:

Among different aldehydes (PDAB, PDAC and Vanillin) tried for developing the colour in alcoholic medium (MeOH), Vanillin was found to be superior for its sensitivity. This method involves the condensation of the **LOP** with Vanillin in acid medium. The effect of various parameters, such as concentration and volume of Vanillin , nature and strength of acid, order of addition of reagents, solvent for final dilution were studied and the optimum conditions developed and actual conditions chosen for the procedure are recorded in (**Table 3**).

3.2.2 Method – M_2 [NIN-AA]

The method involves the reaction between drug and ninhydrin reagent to produce blue color. The conditions were fixed basing on the study of the effects of various parameters such as volume of ninhydrin, nature and conc. of reducing agent, pH and volume of the buffer, heating time and temp, order of addition of the reagents, solvent for final dilution and stability of the colored products after final dilution. The optimum conditions were established by measuring the absorbance's at 560nm and the results are presented in **Table 3.**

3.3 Optical Characteristics:

In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance's at appropriate wave lengths of a set of solutions containing varying amounts of LOP and specified amounts of reagents (as given in the recommended procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded graphically (**Figs. 3 to 4**). Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range (**Table. 3**) for LOP in each method developed. With mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values. (**Table. 3**).

3.4 Precision:

The precision of each proposal methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of LOP in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (**Table 3.**).

3.5 Accuracy:

To determine the accuracy of each proposed method, different amounts of bulk samples of LOP within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in (**Table 3.**).

3.6. Interference studies:

The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of LOP in methods **M_1**, **M_2**, under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

TABLE 3: Optical And Regression Characteristics, Precision And Accuracy Of The Proposed Methods For Lop

Parameter	**M_1**	**M_2**
λ_{max} (nm)	535	535
Beer's law limits (μg/mL)	10.0-50.0	4.0-20.0
Detection limit (μg/mL)	1.5940	0.5352
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	6.335×10^3	1.265×10^4
Sandell's sensitivity ($\mu g.cm^{-2}$/0.001 absorbance unit)	3.441×10^{-2}	0.987×10^{-2}
Optimum photometric range (μg/mL)	1.2-1.8	1.0-1.4
Regression equation (Y=a+bc) slope (b)	0.0101	0.0195
Standard deviation on slope (S_b)	1.619×10^{-4}	2.626×10^{-4}

Intercept (a)	-3.10×10^{-3}	1.44×10^{-3}
Standard deviation on intercept (S_a)	5.378×10^{-3}	3.483×10^{-3}
Standard error on estimation (S_e)	5.121×10^{-3}	3.321×10^{-3}
Correlation coefficient (r)	0.9996	0.9997
Relative standard deviation (%)	1.4813	1.6764
% Range of error (confidence limits)		
0.05 level	1.554	1.759
0.01 level	2.438	2.759

*** Average of six determinations considered**
**** Average of three determinations**

FIG. 1: Absorption Spectrum Of Lop With Vanillin (M_1)

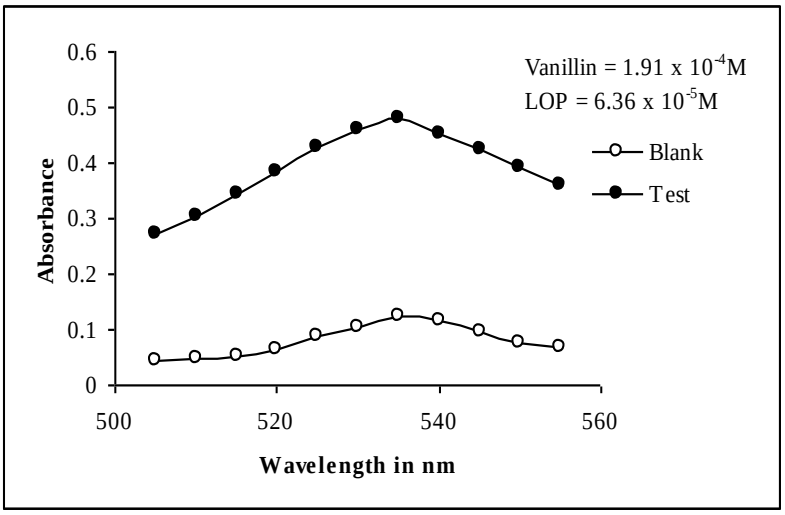


FIG. 2: Absorption Spectrum Of Lop With Ninhydrin – Aa (M_2)

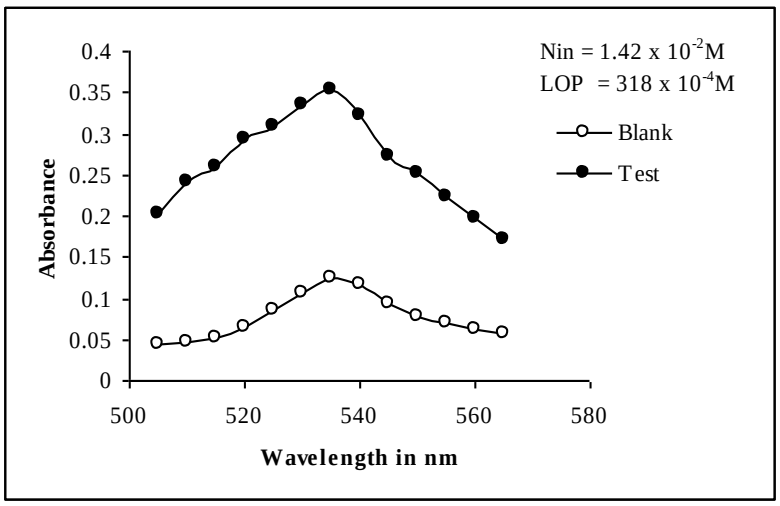


FIG. 3: Beer's Law Plot Of Lop With Vanillin (M_{16})

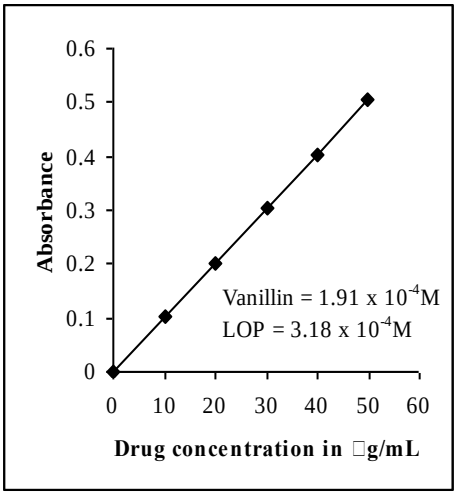
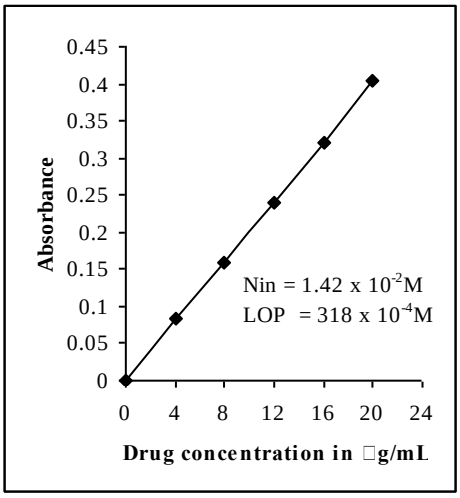


FIG. 4: BEER'S LAW PLOT OF LOP WITH NINHYDRIN – AA (M_{17})



IV. Conclusions

The proposed methods exploit the various functional groups in **LOP** molecule. Statistical analysis of the results shows that the proposed procedures have good precision and accuracy with good sensitivity and higher λ_{max}. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations. The order of $\square_{max}$ among the proposed methods is: $\mathbf{M_1 > M_2}$.

Thus, the proposed methods are simple, sensitive or selective with reasonable precision and accuracy and constitute better alternatives to the reported ones in the assay of **LOP** in bulk form and pharmaceutical formulations.

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