

SPECTROPHOTOMETRIC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF A LAMIVIDINE AND STAVUDINE IN SOLID DOSAGE FORM

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Abstract:

A simple, rapid and UV-Visible Spectrometric method development and Validation for the simultaneous estimation of lamivudine and stavudine in tablet dosage form. The Simultaneous estimation was performed on Shimadzu 1800 double beam UV - Visible spectrophotometer, methanol used as Solvent. Simultaneous equation method was carried out at 271.50 nm (λ_{max} of Lamivudine) and 263.90 nm (λ_{max} of Stavudine). Linearity was observed in range of 3-30 $\mu\text{g/ml}$ for Lamivudine and Stavudine respectively. The correlation coefficient value was found to be 0.9995 & 0.9993 for both drug. Method was statistically validated as per ICH guidelines and can be successively applied for analysis for tablets formulation.. The proposed method was also found to be accurate, precise and robust. The method could be applied to routine quality control of pharmaceutical formulations containing lamivudine and stavudine.

Keywords: Lamivudine, Stavudine, simultaneous equation method.

1 Introduction

From the past studies it was concluded that Lamivudine and Stavudine are emerging as the choice of drug administration for the anti-retroviral agents. Lamivudine is chemically described as (2R,5S)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1H)-pyrimidinone.¹ The antiretroviral activity of Lamivudine is due to its active intracellular anabolite, Lamivudine 5'-triphosphate (Lamivudine-TP)² and Stavudine is chemically described as 2',3',didehydro-3'-deoxythymidine¹ and act as thymidine nucleoside analogue that was active against HIV-1 and HIV-2.² A Lot of analytical methods were found to be developed and validated by using various instrumental methods of analysis. Reverse Phase High Performance Liquid chromatography (RP-HPLC).³⁻⁸ High Performance Thin Layer Chromatography (HPTLC),^{9,10} UV- Visible Spectrophotometry^{8,10}, Liquid Chromatography-

Mass spectroscopy (LC-MS),¹¹⁻¹² chemometrics associated spectrometry,¹³ and others.^{14,15}

The above techniques were developed using the active pharmaceutical ingredients (API), and tablets dosage forms. The point of view was that the methods were developed for Lamivudine and Stavudine as individual drug or in combination with other anti-retroviral agents.

Validation of the method will be done in accordance with USP¹⁷ and ICH¹⁶ guidelines for the assay of active ingredients. The methods will be validated for parameters like accuracy, linearity, precision etc. These methods provide means to simultaneously characterize and quantify the components.

2 Materials and Methods

UV spectrophotometric method was carried out using Shimadzu 1800 double beam UV - Visible spectrophotometer, spectral band width of 2 nm, wavelength accuracy ± 0.5 nm and 1 cm matched pair quartz cells. Standard of

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Lamivudine and Stavudine was obtained from Micro Labs Pvt. Ltd., Bangalore, India as gift sample. All chemicals were used as AR grade methanol for UV method. Lamivir-S-30 was purchased from local pharmacy.

Preparation of Stock Solution: Accurately 10 mg of lamivudine was weighed in to 100 ml clean and dry volumetric flask and 70 ml methanol was added and sonicated for 10 minutes and adjust volume with methanol.

Accurately 10 mg of stavudine was weighed into 100 ml clean and dry volumetric flask, 70 ml of methanol was added, Sonicated for 10 minutes and makeup the volume with methanol. All solutions were freshly prepared prior to analysis.

This stock solution is used for making dilutions for calibration curve.

Determination of λ_{max}

The standard solutions of 100 $\mu\text{g/ml}$ of Lamivudine and Stavudine were individually scanned separately in the range of 200-400nm and the λ_{max} was found to be for both drug reported in Fig-1& Fig-2. The overlain spectrum of both the drugs is also run and recorded as Fig-3.

Calibration Curve.

For each drug appropriate aliquots were pipetted out from standard stock solution into a series of 10 ml volumetric flask and the volume was made up to the mark with methanol to get concentrations of 3-30 $\mu\text{g/ml}$ (n=10) Lamivudine of and 3-30 $\mu\text{g/ml}$ (n=10) Stavudine. For 3 ppm 0.3 ml of stock solution taken and diluted upto 10 ml by solvent and for other as shown in observation table below Respectively Solutions of different concentrations for each drug were scanned at

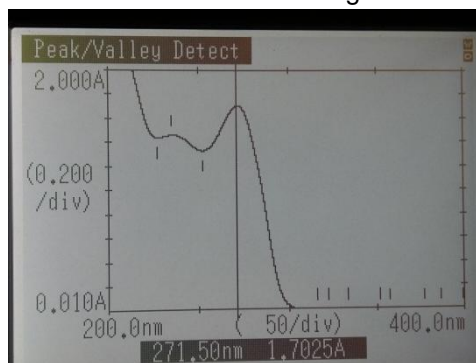


Fig-1- λ_{max} of Lamivudine at 271.50 nm

both λ_{max} and absorbances were recorded. The calculations were done using simultaneous equation method. 10 ml by solvent and for other as shown in observation table below Respectively Solutions of different concentrations for each drug were scanned at both λ_{max} and absorbances were recorded. The calculations were done using simultaneous equation method.

Preparation of Mix Standard Solution.

Accurately 20 mg of lamivudine was weighed in to 200ml clean and dry volumetric flask and added 140 ml methanol. Sonicated for 10 minutes and made volume with methanol. (Solution A).

Accurately 20 mg of stavudine was weighed into 200ml clean and dry volumetric flask, then 10.0ml of solution A was added using A-grade bulb pipette, add 100ml of methanol. Sonicated for 10 minutes and made volume with methanol. (Mix Standard). All solutions were freshly prepared prior to analysis.

Experimental Method

Method -Simultaneous equation method

Two wavelengths selected for the method are 271.50 nm(λ_1) and 263.90 nm(λ_2) that are absorbance maxima of Lamivudine and Stavudine respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 3-30 $\mu\text{g/ml}$ of Lamivudine and 3-30 $\mu\text{g/ml}$ of Stavudine. The absorbances were measured at the selected wavelengths mentioned in table-1 & 2. Concentrations in the sample were obtained by using following equations:

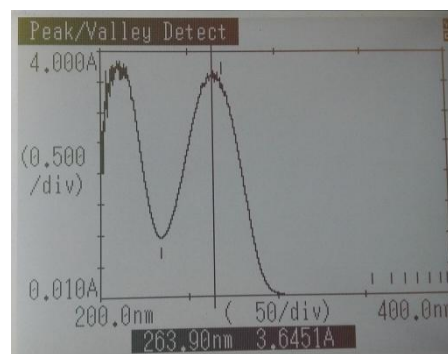


Fig-2- λ_{max} of Stavudine at 263.900 nm

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

C_x and C_y = Concentration of ESC and ETI respectively (gm/100 ml)
 a_{x1} and a_{x2} = Absorptivity of ESC at λ_1 and λ_2 respectively
 a_{y1} and a_{y2} = Absorptivity of ETI at λ_1 and λ_2 respectively
 A_1 and A_2 = Absorbance of test at λ_1 and λ_2 respectively

Table No. 1. Absorbance values for lamivudine

Sr. No.	Stock solution		Conc. of lamivudine ($\mu\text{g/ml}$)	Absorbance at 263.9 nm	Absorbance at 271.5 nm
	ml of stock solution taken	Diluted up to 10 ml			
1	0.3	10	3	0.188	0.464
2	0.6	10	6	0.287	0.589
3	0.9	10	9	0.375	0.728
4	1.2	10	12	0.459	0.843
5	1.5	10	15	0.546	0.978
6	1.8	10	18	0.637	1.091
7	2.1	10	21	0.723	1.213
8	2.4	10	24	0.822	1.343
9	2.7	10	27	0.906	1.446
10	3.0	10	30	1.016	1.578

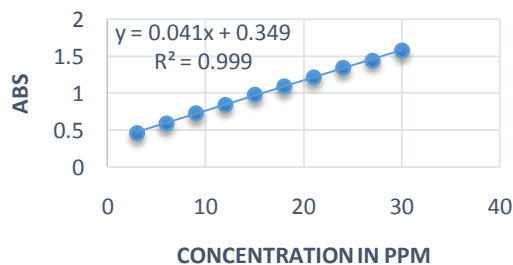
Analytical Method Validation: ^{16, 17}

1) Linearity and Range: The standard solutions of both Lamivudine and Stavudine were scanned in the range of 400-200 nm against solvent methanol and absorbance was measured at λ_{max} of 271.50nm and 263.90nm respectively. The stock solution was diluted with distilled water to reach a concentration range 3-30 $\mu\text{g/ml}$ for both drugs. The absorbance was plotted against the corresponding concentrations to obtain the calibration graphs in Fig-3 and Fig-4.

Table No-2 Absorbance values for Stavudine

Sr. No	Stock solution		Conc. Of Stavudine (g/ml)	Absorbance at 263.9 nm	Absorbance at 271.5 nm
	MI of stock solution taken	Diluted up to 10 ml			
1	0.3	10	3	0.135	1
2	0.6	10	6	0.266	2
3	0.9	10	9	0.398	3
4	1.2	10	12	0.543	4
5	1.5	10	15	0.682	5
6	1.8	10	18	0.825	6
7	2.1	10	21	0.974	7
8	2.4	10	24	1.095	8
9	2.7	10	27	1.206	9
10	3.0	10	30	1.374	10

Lamivudin AT 271.5 NM



Lamivudin AT 263.9 NM

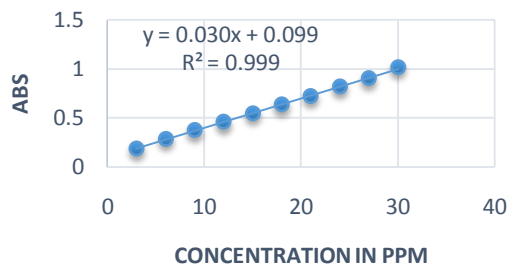


Fig. No. 3: Calibration Curves for Lamivudine at 271.5 nm and 263.9 nm

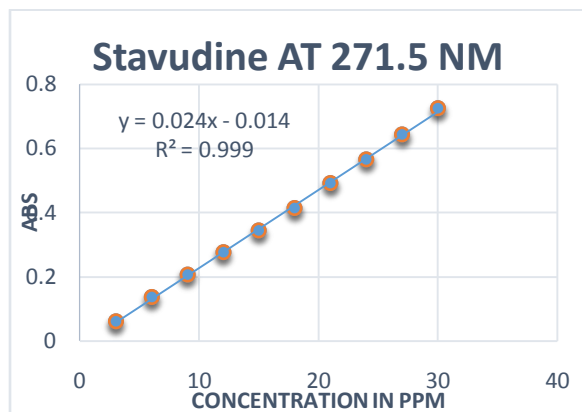
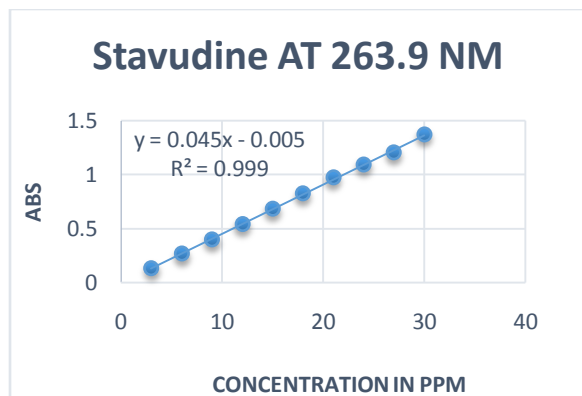


Fig. No- 4 Calibration Curves of Stavudine at 263.9nm & 271.5nm

2) Accuracy- From the total amount of drug found, the percentage recovery was found in 99.99% and 98.79% for Stavudine and Lamivudine Respectively.

3) Precision- The standard solutions of drug sample were prepared and analyzed. The percentage relative standard deviation (RSD %) was found to be 100.1 ± 0.671 and 99.4 ± 0.947 for Stavudine and Lamivudine Respectively.

4) Limit of detection (LOD) and Limit of quantification (LOQ) - LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. The LOD and LOQ for Lamivudine and Stavudine were determined according to ICH guideline as;

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where,

σ = Standard deviation of the y intercept of calibration curves

S = Slope of the calibration curve

The results of LOD and LOQ were shown in table 5.

Results and Discussion

An attempt was made to develop a accurate, simple, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of Lamivudine and stavudine in their combined dosage forms. Both the drugs obey Beer Lambert's law in the range of 3-30 $\mu\text{g/ml}$ at $\lambda\text{-max}$ of 271.50 nm and 263.90 nm for Lamivudine and Stavudine respectively. The simultaneous equation used for analysis of lamivudine and stavudine in sample formulation gives satisfactory results which comply with the label claim of for both drugs was given in table No-3. The method has been further validated for limit of detection, linearity, range, precision and accuracy as given in table No-5. The recovery of drug in tablet formulation was found to be 99.63 ± 0.742 and 99.12 ± 0.738 at 0.744 and 0.743 Coefficient of variance for both drugs. The sensitivity of the method was found to be satisfactory.

The results of Method Development and Validation of Stavudine and Lamivudine including all the analytical data were given as follows:

Table No. 3. Result of Analysis of Tablet Formulation

Formulation	Drug	Label Claim (mg)	% Label Claim *(Mean \pm S.D.)	Coefficient of variance
Tablet-1	Lamivudine	150 mg	99.79 ± 1.78	0.801
	Stavudine	30 mg	98.67 ± 1.021	0.943

SD stands for standard deviation

*Average of six determinations

Table No. 4. Result of Recovery Studies:

Formulation	Drug	Label Claim (mg)	% Label Claim *(Mean \pm S.D.)	Coefficient of variance
Tablet-1	Lamuvudine	150 mg	99.79 \pm 1.78	0.801
	Stavudine	30 mg	98.67 \pm 1.021	0.943

Table No. 5 Result of Analysis of UV method

Parameters	STAVUDINE	LAMUVIDINE
Detection wavelength	263.9nm	271.5nm
Beers law limit	0-30 μ g/ ml	0-30 μ g/ ml
Accuracy	99.99%	98.79%
Precision Repeatability (% R S. D.)	100.1 \pm 0.671	99.4 \pm 0.947
LOD	3.24	3.29
LOQ	9.82	9.97
Regression Equation data:		
Slope	0.0458	0.0411
Intercept	0.0053	0.3499
Correlation coefficient	0.9993	0.9995

Conclusion:

The new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of Lamivudine and Stavudine in pharmaceutical formulations. The

developed methods were validated and from the statistical data, it was found that the method was linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without interference of excipients.

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DECLARATION OF CONFLICT INTEREST

Author declares no conflict of interest.

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