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METHOD DEVELOPMENT AND VALIDATION OF LAMIVUDINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING UV-VIS SPECTROPHOTOMETRIC METHOD

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Abstract

The present study describes a simple, accurate, precise and cost effective UV-Visible spectrophotometric method for the estimation of Lamivudine, an anti HIV drug, in bulk and pharmaceutical dosage form. The solvent used was distilled water and the λ max or the absorption maxima of the drug was found to be 271 nm. A linear response was observed in the range of 10-60µg/ml with a regression coefficient of 0.998. The method was then validated for different parameters as per the ICH (International Conference on Harmonization) guidelines. This method can be used for the determination of Lamivudine in quality control of formulation.

Keywords: Lamivudine, UV-Visible Spectroscopy, λ max.

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INTRODUCTION

Lamivudine, commonly called 3TC, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when others options are not possible. Lamivudine is a nucleotide reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase. It is effective against both HIV-1 and HIV-2. Lamivudine was approved for used in United States in 1995. The aim of this work is to develop and validate an analytical method by using UV- Visible spectro-photometry for the estimation of lamivudine in bulk and pharmaceutical dosage forms. Lamivudine is chemically 4-Amino-1-[2R,5S]-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one [1-5].

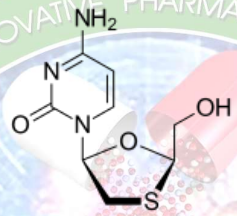

Fig.1: Structure of Lamivudine

MATERIALS AND METHODS

The instrument used for the study was an UV double beam spectro-photometer (Model UV-1800, Shimadzu UV- Visible Spectro - photometer) with 1cm matched pair quartz cells. The solvent used was distilled water.

METHOD DEVELOPMENT

Solubility Test: Solubility test for the drug lamivudine was performed by using various solvents. The solvents include Distilled water, Methanol, Ethanol, Acetonitrile, 0.1N Hydrochloric Acid(HCl), 0.1N Sodium Hydroxide(NaOH). However, Distilled water was chosen as a solvent for developing the method.

DETERMINATION OF λ MAX

Preparation of stock solution: Standard stock solution of lamivudine was prepared by dissolving 10mg of lamivudine in 10ml of distilled water to produce a concentration of 1000µg/ml. 1ml of the stock solution was taken and then diluted up to10ml by using distilled water to produce a concentration of 100µg/ml which is the standard stock solution [6,7].

Preparation of working standard solution: From the above stock solution, 1ml was pipette in to a 10ml volumetric flask and the volume was made up to the mark with distilled water to

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prepare a concentration of 10µg/ml. Then the sample was scanned in UV-Visible spectro-photometer in the range 400-200 nm using distilled water as a blank and the wavelength corresponding to maximum absorbance (λ max) was found to be 271 nm (fig.2).

Preparation of calibration curve: 1ml of the 100 µg/ml solution was diluted to 10ml by using distilled water to produce 10µg/ml solution. 2ml, 3ml, 4ml and 5ml of 100µg/ml solution were diluted to 10ml using distilled water to produce 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentrations ranging from 10-60µg/ml. Then, the calibration curve was plotted by taking concentration on x- axis and absorbance on y-axis (in fig.2). The curve showed linearity in the concentration range of 10-60µg/ml. The correlation coefficient (r^2) was found to be 0.998.

Assay of Lamivudine Tablet (Lamivir-150 mg): A quantity of powder equivalent to 10 mg of lamivudine was taken in a 100 ml volumetric flask and it was dissolved and diluted up to the mark with distilled water. The resultant solution was ultra sonicated for 5 minutes. The solution was then filtered using what-mann filter paper No.40. From the filtrate, appropriate dilutions were made in distilled water to obtain the desired concentration (10µg/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table1.

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Ruggedness, Limit Of Detection (LOD) and Limit Of Quantification (LOQ).

Linearity: Various aliquots were prepared form the stock solution (100µg/ml) ranging from10-60µg/ml. The samples were scanned in UV-Visible spectro-photometer using distilled water as blank. It was found that the selected drug shows linearity between the 10-60µg/ml (table 8).

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation (Lamivir-150mg) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table 3).

Precision: Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, 6 different solutions of same concentration that is

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10µg/ml were prepared and analyzed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by %RSD (table no.5). In the inter-day variation study, solutions of same concentration10µg/ml were prepared and analyzed three times for three consecutive days and the absorbances were noted. The result was indicated by %RSD (table no.6).

Ruggedness: Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by %RSD (table no.7).

Limit of Detection (LOD): The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value (table no.1).

Limit of Quantification (LOQ): The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.1).

RESULTS AND DISCUSSION

The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.28% to 102.5%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 99.2% to 102.5%. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2%. The results of assay showed that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (101.8%). Summary of validation parameters of proposed spectro-photometric method is shown in table1

RESULTS AND DISCUSSION

Table 1: Summary of Validation Result

S.No	Parameter	Result
1	Linearity indicated by correlation	0.998
2	Precision indicated by %RSD	0.3306 %
3	Accuracy indicated by % recovery	99.97-101.14%
4	Limit of Detection	5.34µg/ml
5	Limit of Quantification	16.19µg/ml
6	Range	10-60 µg/ml
7	Linear regression equation	y=0.036x-0.105
8	Assay indicated by % recovery	99.3%

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Table 2: Optimal Characteristics

S. no	Characterization	Optimized values
1	Beer's Law limit (µg/mL)	10-60µg/ml
2	Correlation coefficient	0.998
3	Regression equation(Y*)	y= 0.036x- 0.105
4	Slope (a)	0.036
5	Intercept(b)	0.105

Table 3: Accuracy Readings of Lamivudine

OBSERVATION						
No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Results		
	Formulation	Pure drug		Mean	SD	%RSD
S1	10	8	99.3	99.26	0.0583	0.058
S2	10	8	99.2			
S3	10	8	99.3			
S4	10	10	99.0	99.3	0.26	0 .26
S5	10	10	99.5			
S6	10	10	99.4			
S7	10	12	96.05	96.1	0.062	0.064
S8	10	12	96.1			
S9	10	12	96.2			

Table 4: Precision Readings Showing Repeatability of Lamivudine

S. No	CONCENTRATION (µg/ml)	ABSORBANCE	STATISTICAL ANALYSIS
1	10	0.221	Mean = 0.221 S.D = 0.0017 % RSD = 0.76
2	10	0.223	
3	10	0.223	
4	10	0.221	
5	10	0.221	
6	10	0.224	
7	10	0.224	
8	10	0.221	
9	10	0.222	
10	10	0.221	

Table 5: Intra-Day Precision

S. No	Concentration (µg/ml)	Absorbance			%
		Morning	Afternoon	Evening	
1	10	0.221	0.222	0.221	
2	10	0.221	0.221	0.224	
3	10	0.223	0.222	0.221	
4	10	0.222	0.221	0.223	
5	10	0.221	0.222	0.223	
6	10	0.221	0.223	0.222	
7	10	0.221	0.222	0.223	
8	10	0.222	0.223	0.221	
9	10	0.221	0.222	0.223	
10	% RSD	0.38	0.318	0.49	0.39%

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Table 6: Inter-Day Precision

S. No	Concentration (µg/ml)	Absorbance			Average
		DAY-1	DAY-2	DAY-3	
1	10	0.222	0.222	0.232	0.225

Table 7: Results Showing Ruggedness of Method for Lamivudine

ANALYST-I			ANALYST-2		
Concentration (µg/ml)	Absorbance	Statistical analysis	Concentration (µg/ml)	Absorbance	Statistical analysis
10	0.221	Mean=0.221 SD=0.0004 %RSD=0.180	10	0.223	Mean=0.221 SD=0.00134 %RSD=0.606
10	0.221		10	0.222	
10	0.221		10	0.221	
10	0.222		10	0.221	
10	0.221		10	0.221	
10	0.221		10	0.223	
Room Temperature			**Temp 50^{0}C**		
Concentration (µg/ml)	Absorbance	Statistical analysis	Concentration (µg/ml)	Absorbance	Statistical analysis
10	0.221	Mean=0.221 S.D=0.004 % RSD=0.180	10	0.221	Mean=0.221 S.D=0.00134 % RSD=0.606
10	0.223		10	0.221	
10	0.221		10	0.223	
10	0.221		10	0.221	
10	0.223		10	0.223	
10	0.221		10	0.221	

Limit of Detection (LOD)

The LOD for Lamivudine was found to be 5.34µg/ml.

Limit of Quantification (LOQ)

The LOQ for Lamivudine was found to be 16.19µg/ml.

Preparation of Calibration Curve:

Table 8: Linearity Table Of Lamivudine In Working Standard

S. No	Concentration (µg/ml)	Absorbance
1	10	0.233
2	20	0.629
3	30	0.987
4	40	1.380
5	50	1.732
6	60	2.039

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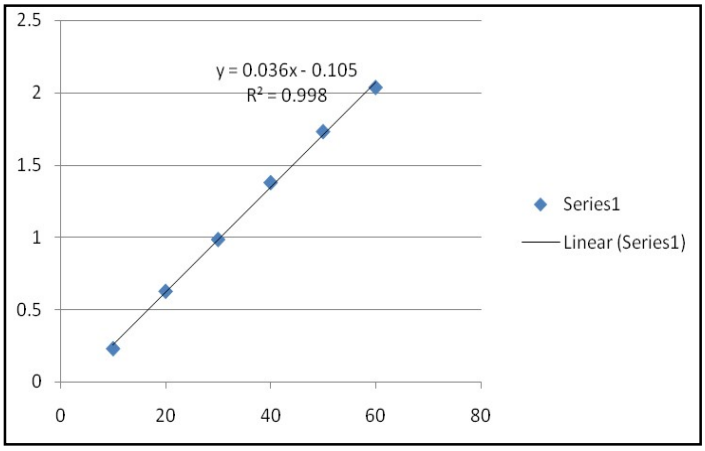

Fig. 3: Calibration Curve Of Lamivudine

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, and cost effective and can be applied successfully for the estimation of lamivudine in bulk and pharmaceutical formulation. The proposed method is also useful for determination of lamivudine stability in sample of pharmaceutical dosage forms.

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