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UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF LAMIVUDINE IN BULK DOSAGE FORM

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ABSTRAC

A simple, accurate and cost efficient UV Spectrophotometric method has been developed and validated for various parameters as per ICH Guidelines for estimation of Lamivudine in bulk powder and tablet dosage form. Distilled water was selected as diluents for proposed method for quantification of Lamivudine in bulk and pharmaceutical dosage form. The Lamivudine showed maximum absorbance at 260nm using distilled water as diluent. The sensitivity of the method is good and also the linearity which is observedover wide concentration range of 2-10 µg/ml. Regression equation and correlationcoefficient values were found to be Y = 0.097x + 0.0012 and R^2 = 0.999, respectively indicating that proposed method was linear. The accuracy method was determined by recovery studies. The recovery of the drug is well within the acceptance limits of 98-102%. The precision was determined by analyzing the samples of Lamivudine. The% RSD for precision was found to be below 2%. The low% RSD values obtained from the analysis indicated that the method was highly precise.

INTRODUCTION

Lamivudine is the nucleoside analogue and reverse transcriptase inhibitor used in the therapy of human immune deficiency virus and hepatitis B virus (HBV) infection. It is available as tablets and oral solution. Literature survey reported that very few analytical methods such as UV Spectroscopy, HPLC have been reported for the estimation of the drug.

MATERIALS AND METHODS

Instrumentation and Materials

The analysis was performed in 10 mm quartz cells using T60U UV-Visible spectrophotometer (PG Instruments Ltd., England) with a fixed 2 nm spectral bandwidth and UV-Win 5 software v 5.1.1 was used for all absorbance measurements.

Methodology solubility studies

Solubility studies for Lamivudine were performed by using various solvents. Phase solubility studies were performed according to the method reported by Higuchi and Connors, 1965. The solubility of the drug was analyzed in distilled water, methanol, and acetonitrile. The excess amounts of drug were transferred in to 10 mL stoppered conical flasks and volume was made up to the mark with respective solvents. The mixture was shaken in thermostatic shaker bath for 24 hr at 37±5 0C. The aliquots of 5 mL were withdrawn and filter through a 0.45-µm. Whatman filter paper for determining drug concentration by UV spectrophotometer.

Selection of solvent

Lamivudine was known to be soluble in solvents like water, methanol, acetonitrile. The absorption pattern of resulting solution is measured against respective blank solution in UV range (200 – 400 nm) and λmax was found in each solvent. The λmax found in each solvent was compared with UV cutoff of the particular solvent to avoid any interaction between the sample peak and solvent peak.

Solubility studies

Determination of absorption maxima: Appropriate volume 0.4 ml of Working Standard solution of Lamivudine was transferred into a 10 ml volumetric flask, diluted to a mark with distilled water to give a concentration of 4µg/ml. the resulting solution was scanned in the UV range (200 – 400 nm). In spectrum Lamivudine showed absorbance maximum at 260 nm.

OPTIMIZED METHOD

Method development for assay of lamivudine

Method development for assay of Lamivudine was initiated based on general method development guidelines and literature.

Preparation of solutions for assay

Preparation of standard solution: The standard solution was prepared by accurately weighing 100 mg of Lamivudine in 100 mL volumetric flask containing 30 mL of diluent and sonicated for about 30 min, and then the volume was made up to the mark with the same diluent to get the final concentration of 1000 µg/mL. From the above solution 10mL was pipette out into a 100

mL volumetric flask and volume was made up to the mark with diluent to get final concentration of 100 µg/mL (working standard solution). Pipette out 1ml from working standard into a clean 10 ml standard volumetric flask and filter through 0.45 µ membrane filter. Make up the final volume with diluent to get final concentration of 10 µg/ml.

Preparation of sample solution

Accurately weighed 20.28 mg of powder was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and marked as sample stock solution.

Pipette out 1ml from sample stock into a clean 10 ml standard volumetric flask and filter through 0.45 µ membrane filter. Make up the final volume with diluent to get final concentration of 10 µg/ml.

Calculation for lamivudine

Assay = 99./

Method validation

1. Specificity

Preparation of lamivudine standard solutions

The standard solution was prepared by accurately weighing 100 mg of Lamivudine in 100 mL volumetric flask containing 30 mL of diluent and sonicated for about 30 min, and then the volume was made up to the mark with the same diluent to get the final concentration of 1000 µg/mL. From the above solution 10 mL was pipette out into a 100 mL volumetric flask and volume was made up to the mark with diluent to get final concentration of 100 µg/mL (working standard solution). Pipette out 0.8 ml from sample stock into a clean 10 ml standard volumetric flask and filter through 0.45 µ membrane filter. Make up the final volume with diluent to get final concentration of 8 µg/ml.

2. Linearity

Preparation of standard solution

The standard solution was prepared by accurately weighing 100 mg of Lamivudine in 100 mL volumetric flask containing 30 mL of diluent and sonicated for about 30 min, and then the volume was made up to the mark with the same diluent to get the final concentration of 1000 µg/mL. From the above solution 10 mL was pipette out into a 100 mL volumetric flask and volume was made up to the mark with diluent to get final concentration of 100 µg/mL (working standard solution).

Preparation of dilutions

From the above working standard solution (100 µg/ml), pipette out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml volumetric flask and volume were made up to the mark with distilled water to get final concentrations of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml.

3. Accuracy

The accuracy was carried out by adding known amounts of standard drug to the analyte (three concentrations levels - 75%, 100% and 125%). At each level, three determinations were performed and the results were recorded.

The accuracy was expressed as percent analyte recovered by the proposed method.

Preparations for accuracy levels

Accuracy Level 1 (50%): 20.05 mg of Lamivudine (equivalent to 20 mg of Lamivudine) reference standard was accurately weighed and transferred into 100 mL volumetric flask. The sample equivalent to 40 mg of Lamivudine was weighed and transferred into the same flask, followed by the addition of 50 mL of diluent. The flask was kept for sonication for 30 minutes and made upto the mark with diluent. From this l0 mL was pipette out and transferred into 100 mL volumetric flask and diluted upto the mark with diluent. Pipette out 1ml from working standard solution into a clean 10 ml standard volumetric flask and filter through 0.45 µ membrane filter. Make up the final volume with diluents to get final concentration of 6 µg/ml.

Accuracy Level 2 (100%): 40.1 mg of Lamivudine (equivalent to 40 mg of Lamivudine) reference standard was accurately weighed and transferred into 100 mL volumetric flask. The sample equivalent to 40 mg of Lamivudine was weighed and transferred into the same flask, followed by the addition of 50 mL of diluent. The flask was kept for sonication for 30 minutes and made up to the mark with diluent. From this l0 mL was pipette out and transferred into 100 mL volumetric flask and diluted up to the mark with diluent. Pipette out 1 ml from working standard solution into a clean 10 ml standard volumetric flask and filter through 0.45 µ membrane filter. Make up the final volume with diluents to get final concentration of 8 µg/ml.

Accuracy Level 3 (150%): 60.15 mg of Lamivudine (equivalent to 60 mg of Lamivudine) reference standard was accurately weighed and transferred into 100 mL volumetric flask. The sample equivalent to 40 mg of Lamivudine was weighed and transferred into the same flask, followed by the addition of 50 mL of diluent. The flask was kept for sonication for 30 minutes and made upto the mark with diluent. From this l0 mL was pipette out and transferred into 100 mL volumetric flask and diluted up to the mark with diluent. Pipette out 1ml from working standard solution into a clean 10ml standard volumetric flask and filter through 0.45 µ membrane filter. Make up the final volume with diluents to get final concentration of 10 µg/ml.

4. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed

conditions. Precision was determined as system precision and method precision, in accordance with ICH guidelines. The results of system and method precision studies were shown in the following table. The low %RSD values obtained from the analysis of tablets indicated that the method was highly precise.

METHOD

System precision

Accurately weighed 10 mg of Lamivudine in 100 ml volumetric flask containing 30 ml of distilled water and sonicated for about 30 min and then the volume was made up to the mark with the same diluent to get the final concentration of 100 µg/ml. From this 0.5 ml was pipetted out into a 10 ml volumetric flask and volume was made up to the mark with diluent to get final concentration of 5 µg/ml and analyzed six times as per test procedure.

Method precision

Accurately weighed and transferred 20.28 mg of fine powder was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent to get final concentration of 100µg/ml. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution. Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

Sample preparation 1: Weigh accurately 20.27 mg of sample was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution 100µg/ml.

Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

Sample preparation 2: Weigh accurately 20.3 mg of sample was transferred to clean
100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution 100µg/ml.

Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

Sample preparation 3: Weigh accurately 20.21 mg of sample was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution 100µg/ml.

Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

Sample preparation 4: Weigh accurately 20.25 mg of sample was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution 100µg/ml.

Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

Sample preparation 5: Weigh accurately 20.23 mg of sample was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution 100µg/ml.

Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

Sample preparation 6: Weigh accurately 20.24 mg of sample was transferred to clean 100 ml standard volumetric flask. Add few ml of diluent and dissolve, make up the final volume with diluent. The solution is sonicated for 30 min and filtered through 0.45 µ membrane filter and marked as sample stock solution 100µg/ml.

Pipette out 0.5 ml from sample stock into a clean 10 ml standard volumetric flask and make up the volume to 10 ml with diluent to get the final concentration of 5µg/ml.

5. Robustness

The following changes were made in the spectroscopic system to determine the effect of deliberate variations in the optimized spectroscopic conditions like wave length, temperature, pH etc.

6. Ruggedness

The ruggedness of an analytical method was the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of test conditions like different analysts, different laboratory, different instrument, different lots of chemical etc. The sample solution of 4µg/mL was prepared in triplicate and their relative absorbance was measured at variable conditions. The % RSD of the results analogous to the absorbance was conveyed.

7. Detection Limit and Quantitation limit

These limits, which are defined based on each experiment, are never "true values", but can only be approximate estimations. Indeed, the calculated values are biased- like all results obtained throughout experiments and will vary from one matrix to a different, from one instrument to another, from one day to another, and from one laboratory to another.

The limits are commonly associated with the signal to noise ratio (S/N). In the case of LOD, analysts often use S/N (signal to noise ratio) of 2:1 or 3:1, while a S/N of 10:1 is often considered to be necessary for the LOQ. Typically, the signal is measured from the base line to peak apex and divided by the peak-to-peak noise, which is determined from the blank plasma injection.

LOQ = 10 σ / S

LOD = 3.3 σ / S

Where, σ - Standard deviation from response S - Slope from calibration curve.

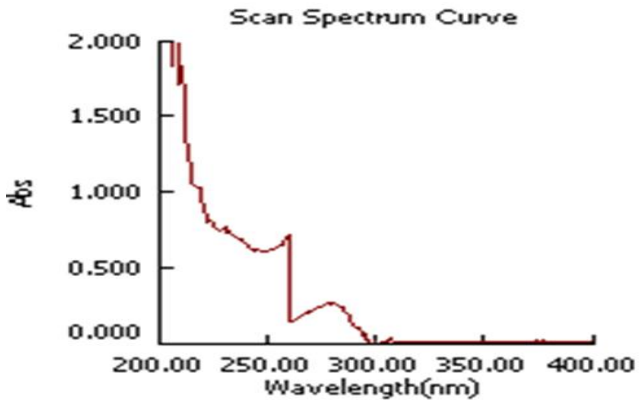
8. Specificity

1. Lamivudine identification

Solutions of Standard and Sample were prepared as per the test method and analysed in the Spectrophotometric system.

Acceptance criteria

Spectrum of standard and sample should be identical.

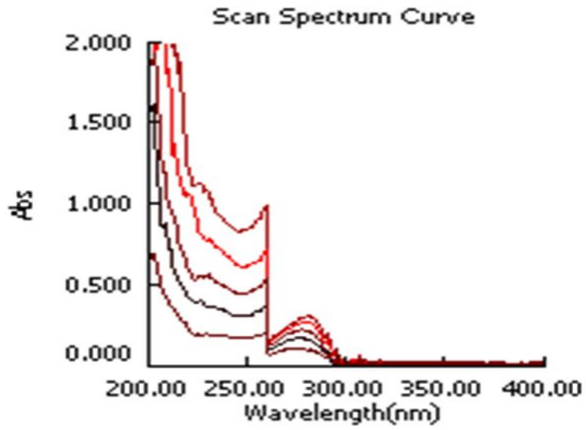


Absorption spectrum of lamivudine standard

2. Linearity

A series of solutions of standard drug substance were prepared in the concentration ranging from 2-10µg/mL Lamivudine a calibration graph is plotted between amount of Concentration Vs Absorbance.

The data obtained in the calibration studies when subjected to linear-regression analysis showed a linear relationship between Concentrations and Absorbance in the range of 2-10µg/mL for Lamivudine. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient. The data of regression analysis and calibration curve were shown in Table .7 and Fig11. Correlation co-efficient (r2) of the linearity study were found to be 0.999 with linear regression equations y = 0.097x+0.001, for Lamivudine, which proves that the method is linear over the Concentration range of drug.



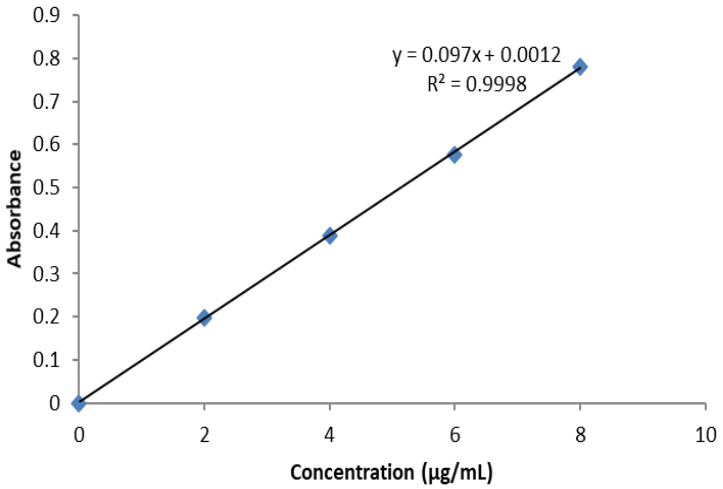
Overlain spectrum of lamivudine

Linearity data of lamivudine

S. No.	Concentration(µg/ml)	Absorbance
1	0	0
2	2	0.199
3	4	0.389
4	6	0.576
5	8	0.781
6	10	0.986

Calibration data of lamivudine

S. No	Parameters	Results
1.	λmax (nm)	260nm
2.	Intercept(a)	0.001
3.	Slope(b)	0.097
4.	Correlation coefficient(r2)	0.999



Calibration curve for lamivudine

3. Accuracy

The accuracy was carried out by adding known amounts of standard drug to the analyte (three concentrations levels - 50, 100 and 150 % - of the labeled claim). At each level, three determinations were performed and the results were recorded. The accuracy was expressed as percent analyte recovered by the proposed method.

S. No	Spiked level	Absorbance	% Recovery	S. D	%RSD	Mean Recovery
1	50% 50% 50%	0.567 0.564 0.565	101.5 100.0 100.5	0.001528	0.27	100.6
2	100% 100% 100%	0.753 0.756 0.758	98.7 99.2 100.0	0.002517	0.33	99.3
3	150% 150% 150%	0.966 0.954 0.961	102.4 100.3 101.5	0.006028	0.62	101.4

Accuracy level 150% of lamivudine

Accuracy data of lamivudine

From the accuracy table it was found that recovery value of drug was in the range 98-102%, which indicates that the method is accurate.

4. Precision

Precision was determined as system precision and method precision, in accordance with ICH guidelines. The results of system and method precision studies were shown in the following table. The low %RSD values obtained from the analysis of tablets indicated that the method was highly precise.

System precision

The system precision was established by injecting six replicate injections of standard solution in to the chromatographic system by maintaining the optimized Spectroscopic conditions.

Method precision

Six samples of drug product of 5 µg/ml of the working test concentration were prepared and injected into the Spectroscopic system.

Acceptance criteria

The individual assays of Lamivudine should be within 98% to 102%.

System precision data of lamivudine

S. no.	Wave Length	Absorbance
1	260	0.452
2	260	0.440
3	260	0.443
4	260	0.442
5	260	0.434
6	260	0.451
Mean	-	0.443
S. D	-	0.006831
%RSD	-	1.541

Method precision data of lamivudine

S. no.	Wave Length	Absorbance	%Assay
1	260	0.432	97.9
2	260	0.439	99.5
3	260	0.441	100
4	260	0.438	99.3
5	260	0.442	100.2
6	260	0.436	98.8
Mean	-	0.438	99.28
S. D	-	0.003633	0.842417
%RSD	-	0.82	0.84

% Assays and % RSD of Assay are within limit, hence the method passes repeatability.

5. Robustness

The following changes were made in the spectroscopic system to determine the effect of deliberate variations in the optimized spectroscopic conditions like wave length, Temperature etc.

Robustness data change in wave length of lamivudine

S. No.	Changeinwave length	Absorbance	%RSD
1	Low (259nm)	0.448	0.883
2	Original (260nm)	0.452	0.883
3	High(261nm)	0.456	0.883

Robustness data change in temperature of lamivudine

S. no.	Changein Temperature	Absorbance	%RSD
1	Low(20°C)	0.456	0.98
2	Original (25°C)	0.461	0.98
3	High(30°C)	0.465	0.98

6. Ruggedness:

The ruggedness of an analytical method was the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of test conditions like different analysts, different laboratory, different instrument, different lots of chemical etc. The sample solution of 4 µg/mL was prepared in triplicate and their relative absorbance was measured at variable conditions. The % RSD of the results analogous to the absorbance was conveyed.

Ruggedness data of lamivudine

DifferentAnalysts	Absorbance	%RSD
Analyst-1 (Ms. B. Roopa)	0.354	0.83
	0.358	
	0.355	
Analyst-2 (Ms.Prashanthi)	0.362	
	0.358	
	0.360	
DifferentLabs:		
Lab – 1 (PA &QA Lab)	0.366	1.1
	0.364	
	0.362	
Lab – 2(UGAnalysis Lab)	0.361	
	0.354	
	0.361	
Differentinstruments:		
Instrument – 1(PGInstrumentT60)	0.339	0.66
	0.344	
	0.342	
Instrument–2(Lab India)	0.341	
	0.345	
	0.344	

7. Detection Limit and Quantitation limit

These limits, which are defined based on each experiment, are never "true values", but can only be approximate estimations. Indeed, the calculated values are biased- like all resultsobtained throughout experiments and will vary from one matrix to a different, from one instrument to another, from one day to another, and from one laboratory to another.

The limits are commonly associated with the signal to noise ratio (S/N). In the case of LOD, analysts often use S/N (signal to noise ratio) of 2:1 or 3:1, while a S/N of 10:1 is often considered to be necessary for the LOQ. Typically, the signal is measured from the base line to peak apex and divided by the peak-to-peak noise, which is determined from the blank plasma injection.

$LOD = 3.3\sigma / S$

$= 3.3\times 0.000492 / 0.097$

$= 0.0167 \mu g/ml$

$LOQ = 10\ \sigma / S$

$= 10 \times 0.000492 / 0.097$

$= 0.0507\ \mu g/ml$

Where, σ - Standard deviation from response S - Slope from calibration curve.

Detection Limit and Quantitation limit data of lamivudine

Parameter	Lamivudine
Detection Limit (LOD)	0.0167
Limit and Quantitation (LOQ)	0.0507

The Proposed study describes a new UV - Spectrophotometric method development and validation of simultaneous estimation of Lamivudine using simple mobile phase. The method gives good resolution of the compound with a short analysis time. The method was validated for System suitable parameters, precision, linearity, accuracy, precision, robustness, ruggedness, LOD, LOQ and assayaccording to ICH guidelines were found to be within limits were postulated in the following table.

RESULTS IN METHOD VALIDATION

Parameters	Results	Acceptancelimits
	Lamivudine	
Specificity	Nointerference	Nointerference
Linearity	0.999	R^2=0.999
Accuracy	99-100%	98-102%
Precision	Rsd<2%	Rsd<2%
Robustness	Rsd<2%	Rsd<2%
Limit ofdetection	0.01	Signal noise ratioshouldbemorethan 3:1
Limit ofquantitation	0.05	Signal noise ratioshouldbemorethan 10:1
Assay	99.1%	98-102%

SUMMARY AND CONCLUSION

For routine analytical purposes it is always of interest to establish methods capable of analyzing a sample in a short time period with due accuracy and precision. The main purpose of the study was to develop specific, accurate, precise and economic methods for the determination of Lamivudine.

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