International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development and Validation of Q-Absorbance Ratio Spectrophotometric Method for the simultaneous Estimation of Lamivudine and Zidovudine in Bulk and Formulation

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Abstract

The present research work demonstrates an analytical method development for simultaneous estimation of Lamivudine andZidovudinein combined dosage form using Q-absorbance ratio concept. While method development, two different wavelengths one representingiso-absorptive point (271 nm) and other representing the λ_{max} of Lamivudine (269 nm) were used. Optimum response was obtained in solvent system that comprisesmethanol and water in ratio of 60:40 v/v.Proposed UV method was found to be linear over the concentration range of 1-20 µg/ml for Lamivudineand that of 1-16 µg/ml for Zidovudine.On the basis of recovery studies after standard addition, accuracy of proposed method was found to be in between 98.12 to 100.61 and 99.10 to 100.96 % for Lamivudine and Zidovudine respectively. Intra-day precision of the method in terms of % relative standard deviation was found to be in between 0.1781 to 1.37 and 0.1325to 1.96 for Lamivudine and Zidovudine respectively. Inter-day precision range of the method for Lamivudine and Zidovudine was found to be in between 0.13 to 1.94 and 0.11 to 1.58 respectively.LODand LOQ of proposed UV method were 0.0451 and 0.1024 µg/ml for Lamivudine and 0.0502 and 0.3835 µg/ml forZidovudine.Proposed UV method was robust and rugged in nature.Proposed methodwas successfully used for the estimation of Lamivudine and Zidovudine contents of in-house formulation consisting of APIs and the common excipients.

Keywords: UV- visible spectrometry, Q absorbance ratio, Lamivudine, Zidovudine, Validation

1. INTRODUCTION:

The advent of antiretroviral therapy (ART) has revolutionized the management of HIV/AIDS, transforming what was once a fatal diagnosis into a chronic yet manageable condition. Among the cornerstone medications within the arsenal of antiretrovirals are Lamivudine and Zidovudine[1-2]. Lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI), and Zidovudine, the first nucleoside analog approved for clinical use, have played pivotal roles in the treatment and prevention of HIV infection[3-5]. Since their approval by regulatory agencies, these drugs have been extensively studied,

both individually and in combination with other agents, demonstrating remarkable efficacy in suppressing viral replication, improving immunological function, and prolonging the lives of individuals living with HIV/AIDS[6-8].

Considering therapeutic and commercial importance of combination of Lamivudine and Zidovudine, it was envisaged that development of UV-Visible spectrophotometric method for simultaneous estimation of Lamivudine and Zidovudine will be worth.

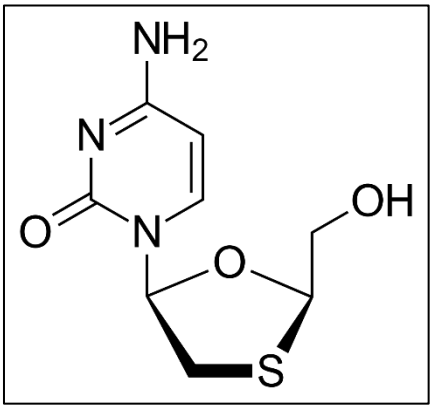

Fig 1. Chemical structure of Lamivudine

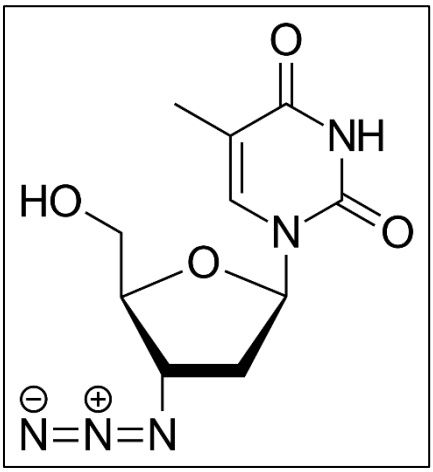

Fig 2. Chemical structure of Zidovudine

2. EXPERIMENTAL:

2.1 Instrumentation:

A double beam UV-visible spectrophotometer (V-530, Jasco) with spectra manager software was used for the method development and validation. Matched quartz cells with 3 cm height and 1 cm path length were used for spectral measurements. Analytical balance (Vibra HT, Essae) was used for the weighing purpose.

2.2 Material and Methods:

All chemicals and reagents used for the method development purpose were of analytical or HPLC grade. Lamivudine and Zidovudinestandard was purchased from theTCI chemicals (INDIA) pvt.ltd.

2.3 Preparation of standard stock solution:

Lamivudine and Zidovudinewas weighed separately (10 mg each) and transferred to the 10 ml pre-calibrated volumetric flasks and dissolved in 5 mlmixture of methanol and water (60:40v/v) to achieve a stock solution of 1000 µg/ml (Stock-1). Stock 1 was suitably diluted to achieve solution of 100µg/ml (stock 2).

Determination of maximum wavelength (λ_{max}):

Stock-2 of Lamivudine and Zidovudine was diluted suitably so as to obtain solutions of 10µg/ml strength. Resultant Lamivudine and Zidovudine solutions were scanned over wavelength range of 800 to 200 nm using medium scanning speed. Obtained spectra were analyzed using Spectra Manager software and the λ_{max} were identified.

Preparation of calibration curve:

Stock 2 of Lamivudine was diluted suitably so as to achieve seven different calibration standards representing 1, 2, 4, 6, 10, 12 and 16µg/ml strength whereas Stock 2 of ZIDOVUDINE was diluted to obtain calibration standards with 1, 2, 4, 6, 12, 16, 20 µg/ml strength. From the full spectrum measurement mode (Figure 3 and 4) of stock-2 of Lamivudine and Zidovudine,two different wavelengths viz. 271 nm and 267 nm were identified as λ_{max}. The calibration curves representing concentration vs. absorbance were plotted (Figure 3 and Figure 4 respectively).

UV-spectrophotometric method:

Q-Absorption ratio analysis method:

Q-Absorption ratio method comprises use the ratio of absorption at two selected wavelengths (one representingiso-absorptive point and other representingλ_{max} of one of the two components). Proposed method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbance at any two wavelengths is a constant value, independent of concentration and path length. The solutions of 10µg/ml and 12µg/ml for Lamivudine and Zidovudine were scanned in the wavelength range of 400 to 200nm to obtain overlain spectra (fig 5). Two wavelengths, 271nm as iso-absorptive point and 269nm (λmax ofLamivudine) were selected for the formation of Q-absorbance ratio equation.

The concentration of the individual components was calculated by using the following equations;

Cx = Qm-Qy/Qx-Qy) ×A1/ax 1(Eqn.3)

Cy = Qm-Qy/Qy-Qx) ×A1 /ax1(Eqn.4)

WhereQm = A2 /A1, A 1 is absorbance of sample at iso-absorptive point,

A2 is absorbance of sample at λ_{max} of one of the two components,

Qx = ax2 /ax1,Qy = ay2 /ay1,

ax 1and ax 2 represent absorptivities of Lamivudine at λ1 and λ2,

ay 1 and ay 2 denote absorptivities of Zidovudine at λ1 and λ2 respectively;

Cx and Cy be the concentration of Lamivudine and Zidovudine respectively.

Validation of UV- visible spectrophotometric methods[9-12]

The developed method for simultaneous estimation of Lamivudine and Zidovudine was validated as per ICH guidelines. Different parameters like linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ) were evaluated.

Linearity and Range:

Linearity of the proposed UV method was established using seven different CAL STDs of Lamivudine and Zidovudine. CAL STDs of Lamivudine and Zidovudine were analyzed at respective wavelengths of maximum absorbance. Calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis.R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy:

Accuracy may often be expressed as % recovery by the assay of known added amount of analyte. To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%) of its predefined concentration. To the predefined concentrations, different amounts of Lamivudine and Zidovudine were added (standard addition method) and the accuracy was calculated on the basis of percent recovery. For calculating the percent recovery following formula was used.

% RC= (SPS-S/SP) × 100

Where,

SPS = Amount found in the spiked sample

S = Amount found in the sample

SP = Amount added to the sample

% RC = Percent recovery

Precision (Inter-day and Intra-day precision):

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of predefined samples. The study was performed at three concentration levels(Lamivudine: 1, 8 and 16µg/ml and Zidovudine: 1,10 and 20 µg/ml). Samples (n=5) were analyzed at three different time intervals of a day. Study was repeated on three consecutive days. Deviation in the results was calculated in terms of % relative standard deviation (% RSD).

Robustness:

Robustness of the method was assessed by analyzing MQC STDs of Lamivudine and Zidovudine (10µg/ml each) at ±1nm of pre-identified wavelength of maximum absorbance for both Lamivudine and Zidovudine. The results were calculated in terms of % RSD.

Ruggedness:

Ruggedness of the method was established by analyzing triplicate samples of Lamivudine and Zidovudine (10µg/ml each) on two different UV-Visible spectrophotometers viz. V-530, Jasco and BA-UV-2600, Bio age. Results were expressed in terms of % RSD.

Limit of Detection and Quantification:

To determine the limit of detection and quantification (LOD and LOQ), the standard deviations (σ) of response and slope of calibration curve (S) were used. Detection of limit was calculated by (3.3×σ/S) and quantification limit was calculated by (10×σ/S).

Estimation ofLamivudine and Zidovudine content in pharmaceutical formulation:

The lamivudine and zidovudine content in its marketed formulation was estimated using pre-validated UV-Visible spectrophotometric method. Tablet formulation contents (labeled strength: 150 and 300 mg/Tablet lamivudine and zidovudine respectively) were dissolved in 100 ml of volumetric flask with co-solvent system (n=5). Said solution was suitably diluted with co-solvent system and obtained solution was filtered through 0.45 µm nylonsyringe filter. Filtered solution was suitably diluted and analyzed for Lamivudine and Zidovudine content by using proposed UV-Visible spectrophotometric method.

Results and Discussion:

Determination of wavelength of maximum absorbance (λ_{max}):

Identification of wavelength having maximum absorbance is prerequisite for quantitative UV analysis. Solution with absorbance value less than 1 were considered to be appropriate for the determination of wavelength having maximum absorbance. Considering the above-mentioned point determination of λmax of Lamivudine and Zidovudine solution of 10 µg/ml concentration each were carried out by full scan mode of UV-Visible spectrophotometer. The full scan mode was processed by Jasco UV software and λmax were determined. The λmax was found to be 271 nm and 267 nm for Lamivudine and Zidovudine (Fig. 3 and Fig. 4) respectively. The overlain spectra of both drugs shown in Fig. 5. The two wavelengths were used for the analysis of the drugs were 271 nm (Iso-absorptive point) and 269 nm (λmax of Lamivudine) at which the calibration curves were prepared for both the drugs.

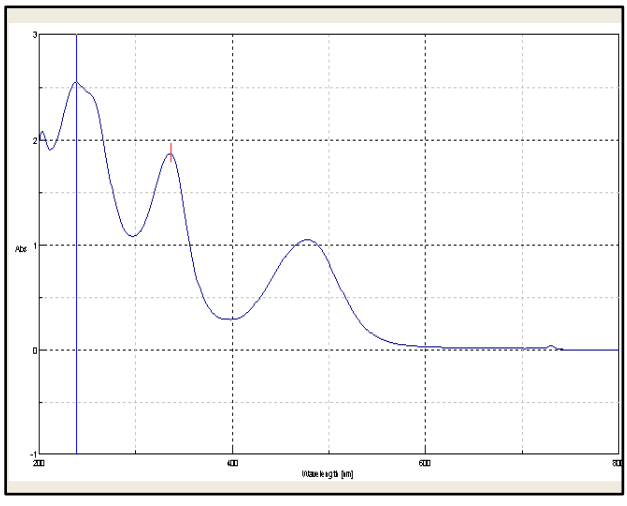

Fig 3. UV-visible spectra of Lamivudine (271 nm)

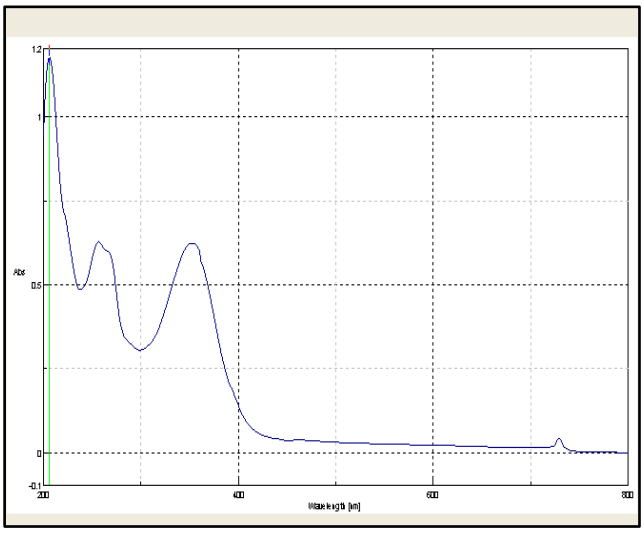

Fig 4.UV-visible spectra of Zidovudine (267 nm)

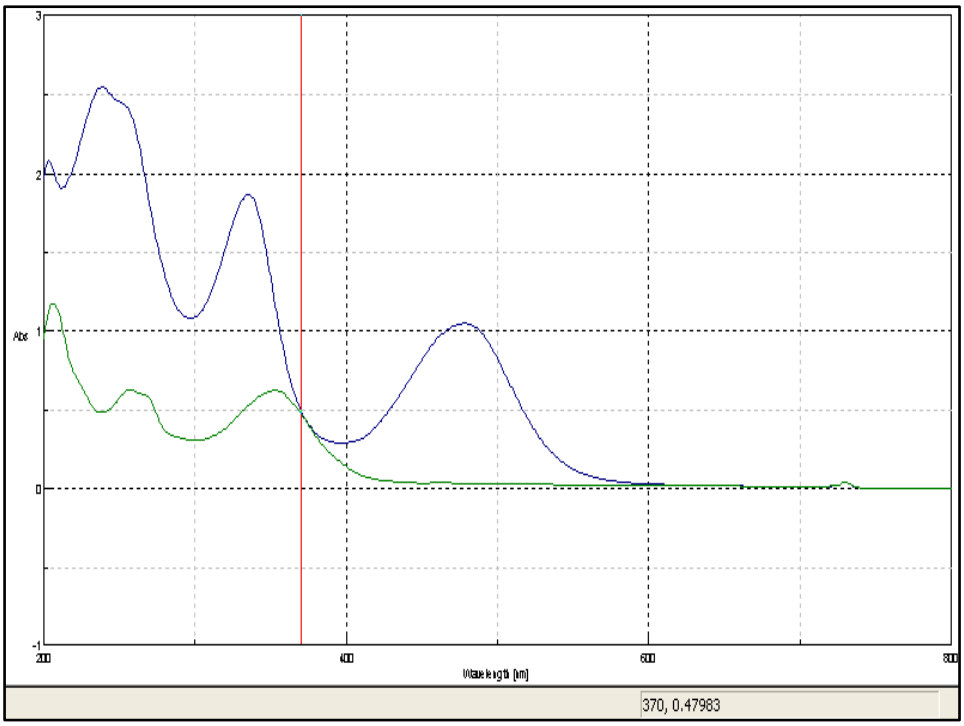

Fig 5. Overlain spectra of Lamivudine and Zidovudine

Preparation of Calibration Curve:

(A) Calibration Curve for Lamivudine:

Calibration curve for Lamivudine consists of different concentrations of standard solution ranging from 1 -16µg/ml. The solutions were prepared by pipetting out 1, 2, 4, 6, 10, 12, 16 µg/ml of the working standard solution of Lamivudine (100µg/ml) into series of 5 ml volumetric flasks and the volume was adjusted to mark with solvent. The absorbance of the solutions was measured at 271nmand 269 nm against solvent ratio of methanol: water (60:40) as a blank.Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity. (Figure 6)

(B) Calibration Curve for Zidovudine:

Calibration curve for zidovudine consists of different concentrations of standard solution ranging from 1-20µg/ml. The solutions were prepared by pipetting out 1, 2, 4, 6, 12, 16, 20 µg/ml of the working standard solution of zidovudine (100µg/ml) into series of 5 ml volumetric flasks and the volume was adjusted to mark with solvent ratio. The absorbance of the solutions was measured at 239 nm and 370nm against solvent ratio of methanol: water (60:40) as a blank.

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity. (Figure 6)

Method validation

Linearity and Range

Linearity and range are the key parameters of analytical method which demonstrates the limit within the intended method to be used for its optimum performance. Considering the importance of linearity and the range, six points calibration curves of lamivudine between the range 1-16µg/ml and zidovudine between the range 1-20µg/ml were plotted. The concentrations and the respective mean absorbance values of Lamivudine and Zidovudine are mentioned in (Table 1 & Table 2). Calibration curve was subjected to least square regression analysis yielded an equation; y = 0.074X + 0.003 and y = 0.041X+ 0.003 with correlation coefficient for Lamivudine and Zidovudine in 271nm respectively (Fig. 6 and and other to least square regression analysis yielded an equation; y = 0.051X + 0.003 and y = 0.049X +0.001 with correlation coefficient for Lamivudine and Zidovudine in 269nm respectively (Fig. 7). The linearity study revealed that the developed UV method was found to be linear adherence to the system of Beers Law over the concentration range of 1 to 16 µg/ml for lamivudineand 1 to 20 µg/ml zidovudine.

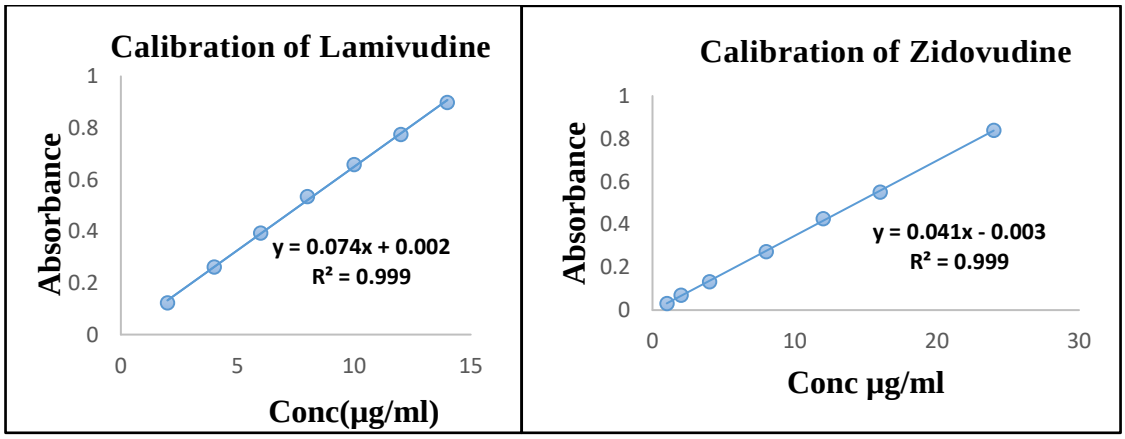

Fig 6. Calibration curve of Lamivudine and Zidovudine at 271 nm

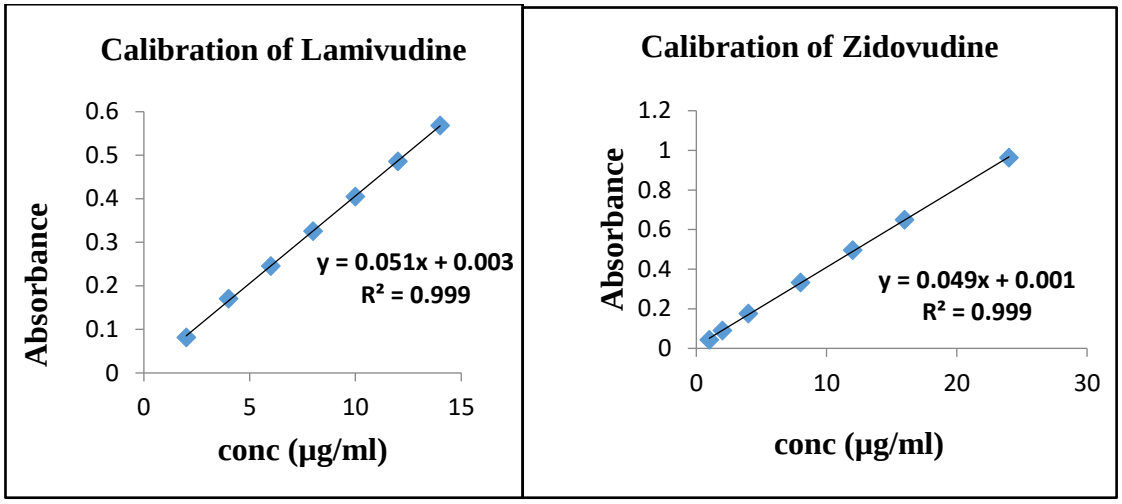

Fig 7. Calibration curve of Lamivudine and Zidovudine at 269 nm

Table 1: Calibration data at λmax (271nm)

	Lamivudine		**Zidovudine**	
Sr No.	**Con (µg/ml)**	**Absorbance**	**Con (µg/ml)**	**Absorbance**
1	1	0.1137	1	0.0401
2	2	0.2731	2	0.0821
3	4	0.3816	4	0.1221
4	6	0.5188	6	0.2835
5	10	0.6625	12	0.4263
6	12	0.7825	16	0.5523
7	16	0.9092	20	0.8421

Table 2: Calibration data at Iso absorptive Point (370nm)

	Lamivudine		**Zidovudine**	
Sr No.	Con (µg/ml)	Absorbance	Con (µg/ml)	Absorbance
1	1	0.0714	1	0.0422
2	2	0.1695	2	0.0901
3	4	0.2273	4	0.1753
4	6	0.3589	6	0.3343
5	10	0.4106	12	0.5007
6	12	0.4835	16	0.6521
7	16	0.5714	20	0.9768

Accuracy:

Accuracy is the measure of how close the experimental value is to the true value. The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value. sometimes it termed as trueness. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. UV method for lamivudine and zidovudine, accuracy was established by recovery studies.The results of accuracy studies, determined that thedeveloped UV method is highly accurate as the percent recovery was found to be between 98.12 to 100.23% (Table 3).

Table 3: Recovery studies for lamivudine and zidovudine

Origin level (µg/ml)	Lamivudine				Origin level (µg/ml)	Zidovudine			
	Conc.(%)	Amount Added	% Recovery	% RSD		Conc (%)	Amount Added	% Recovery	% RSD
10	80	8	98.12	0.1786	10	80	0.8	99.73	0.3414
10	100	10	99.03	0.1197	10	100	10	100.23	0.1137
10	120	12	100.61	0.4325	10	120	28.8	99.10	0.1940

Precision:

Precision is the variability among replicate measurements, i.e., how close the values in a series of results are to each other. Precision of the assay was determined by repeatability and intermediate precision, which was studied by comparing the assays on 3 different days. It is expected that an analytical method should generate reproducible outcomes. Precise analytical method leads to accurate results. Considering the importance of reproducible and accurate results, Inter-day, intra-day variations were studied to determine repeatability and intermediate precision of the proposed analytical method. Intermediate precision was determined by analyzing three different levels of Lamivudine and Zidovudine concentrations at 1, 8, 16 and 1, 10, 20 µg/ml respectively.The results were expressed in terms of mean absorbance values, percent assay and % RSD for the intra-day and inter-day precision study, demonstrated in Table 4-7, respectively for lamivudine and zidovudine. % RSD values were less than 2, demonstrated the precision of developed UV method.

Table 4: Intra-day precision data of UV method for Lamivudine

Sr. No			Morning			Afternoon			Evening		
	Wavelength (nm)	Conc (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD

1	271	1	0.1141	99.16	0.220	0.1045	98.71	0.782	0.1280	101.28	0.213
	269		0.0774	100.12	0.2639	0.0969	99.25	0.199	0.0978	100.12	0.247
2	271	8	0.5144	99.32	1.0294	0.5182	99.92	0.963	0.5161	99.25	0.434
	269		0.3394	100.24	1.3708	0.3175	99.16	0.516	0.3470	100.19	0.844
3	271	16	0.8930	99.95	0.4935	0.9115	100.24	0.187	0.9118	100.08	0.177
	269		0.5729	100.17	0.7008	0.5760	99.34	0.346	0.5826	100.19	1.044

Table 5: Inter-day precision data of UV method for Lamivudine

Sr. NO	Wavelength (nm)	Conc (µg/ml)	Day 1			Day 2			Day 3		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	271	1	0.1067	100.55	1.9537	0.1257	99.65	0.4854	0.1276	101.13	1.0331
	269		0.0876	100.14	0.6201	0.0881	101.18	0.1317	0.0874	101.03	0.1759
2	271	10	0.5288	100.6	0.3658	0.5292	100.70	0.3869	0.5306	101.10	0.2874
	269		0.3296	100.51	0.3879	0.3290	100.33	0.8492	0.3286	100.42	0.3899
3	271	20	0.9049	100.53	0.2362	0.9028	100.28	0.2613	0.9045	100.49	0.2030
	269		0.5731	100.57	0.6382	0.5749	100.89	0.9347	0.5739	100.71	0.8050

Table 6: Intra-day precision data of UV method for zidovudine

Sr. NO			Morning			Afternoon			Evening		
	Wavelength (nm)	Conc (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD

1	271	1	0.1148	99.75	0.2213	0.1051	99.30	0.7867	0.1288	101.89	0.2143
	269		0.0779	100.72	0.2655	0.0975	99.85	0.2002	0.0984	100.72	0.2485
2	271	8	0.5175	99.92	1.0356	0.5213	100.52	0.9688	0.5192	99.85	0.4366
	269		0.3414	100.84	1.3790	0.3194	99.75	0.5191	0.3491	100.79	0.8491
3	271	16	0.8984	100.55	0.4965	0.9170	100.84	0.1881	0.9173	100.68	0.1781
	269		0.5763	100.77	0.7050	0.5795	99.94	0.3481	0.5861	100.79	1.0503

Table 7: Inter-day precision data of UV method for zidovudine

Sr. NO	Wavelength (nm)	Conc (µg/ml)	Day 1			Day 2			Day 30		
			Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	271	1	0.1073	101.15	1.9654	0.1265	100.25	0.4883	0.1284	101.74	1.0393
	269		0.0881	100.74	0.6238	0.0886	101.79	0.1325	0.0879	101.64	0.1770
2	271	10	0.5320	101.20	0.3680	0.5324	101.30	0.3892	0.5338	101.71	0.2891
	269		0.3316	101.11	0.3902	0.3310	100.93	0.8543	0.3306	101.02	0.3922
3	271	20	0.9103	101.13	0.2376	0.9082	100.88	0.2629	0.9099	101.09	0.2042
	269		0.5765	101.17	0.6420	0.5783	101.50	0.9403	0.5773	101.31	0.8098

Robustness

Robustness examines the effect that operational parameters such as temperature, mobile phase composition, detection wavelength etc, have on the analysis results. If the influence of parameter is said to be within a previously specified tolerance, the parameter is said to be within the methods robustness range. Robustnessstudy of proposed UV method was evaluated by using three different solvents. The method was found to be robust as indicated by the % RSD values which are less than 2%. The% RSD values were found to be between0.1131and0.8961 for lamivudine and between 0.3965 and 0.6314forzidovudine, shown in Table 8 for lamivudine and zidovudine respectively. Percentage RSD values were below 2 depict that the proposed UV method was robust in nature.

Table 8: Robustness study for Lamivudine and Zidovudine

Conc. (µg/ml)	Lamivudine			Conc. (µg/ml)	Zidovudine		
	λmax	Absorbance Mean	% RSD		λmax	Absorbance Mean	%RSD
10	271	0.6411	0.1131	10	271	0.6621	0.3965
10	269	0.4215	0.8961	10	269	0.4132	0.6314

Ruggedness:

Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different instruments, different elapsed assay times, different assay temperatures, different days etc.Ruggednes analytical methods are free from environmental/external factors impact. The ruggedness of proposed UV method, for lamivudine and zidovudine solutions were analysed by using two different UV-Visible spectrophotometers. Sample analysis resulted into % RSD values between 0.4175 and 0.9113 for Lamivudineand between 0.3732 and 0.6432 for Zidovudine. Results showed that the proposed UV method was rugged as % RSD values were less than 2, shown in Table 9.

Table 9: Ruggedness study for Lamivudine and Zidovudine

Sr.	Make of Instrument	Theoretical Conc (µg/ml)	% RSD	
			Lamivudine	Zidovudine
1	V-530, Jasco	10	0.4175	0.3732
2	UV-2600, Bio age	10	0.9113	0.6432

Limit of Quantitation (LOQ) and Limit of Detection (LOD):

Generally, LOQ is the first calibration standard. LOQ represents the lowermost concentration that can be analysed. LOD represents the lowest quantity of substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). LOD and LOQ of proposed UV method were found to be 0.0451 and 0.1024 µg/ml for lamivudine whereas 0.0502 and 0.3835 µg/ml for zidovudine, as shown in Table 10 for lamivudineand zidovudine. Lower LOQ values indicated that the proposed method would be sensitive enough to quantify the Lamivudine and Zidovudine content of samples at its lower level.

Table 10: LOD and LOQ for Lamivudine and Zidovudine

Sr. No.	Parameter	Lamivudine (µg/ml)	Zidovudine (µg/ml)
1	LOD	0.0451	0.0502
2	LOQ	0.1024	0.3835

Estimation of Lamivudine and Zidovudine content in pharmaceutical formulation:

The developed UV method was successfully applied for estimation of Lamivudine and Zidovudine content in pharmaceutical formulation. The Lamivudine and Zidovudine content in the pharmaceutical formulation was found to be 99.70 % and 99.5% respectively (Table no 11) by Q-Absorbance method**.**

Table 11: Analysis of content in pharmaceutical formulation

Sr no.	Sample (n=5)	Amount present(µg/ml)	Amount found(µg/ml)	Assay%
1	Lamivudine	10	9.97	99.70
2	Zidovudine	10	9.95	99.5

Conclusion:

The simple, precise, accurate, and sensitive UV- visible spectrophotometric method for the Q Absorbance of Lamivudine and Zidovudine in a bulk drug and pharmaceutical formulation was developed and validated. The recovery result confirms the accuracy of method. The proposed method was found to be robust and rugged in nature. Thus, it can be effectively applied for the estimation of Lamivudine and Zidovudine in pharmaceutical formulation.

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