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Research Article

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT & VALIDATION ON SUGAR DERIVATIVES OF LAMIVUDINE

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ABSTRACT

The present study includes a simple, sensitive and specific UV method development and validation for the quantitation of lamivudine dextrose & lamivudine xylose. The λmax was found to be 271 nm by taking water as solvent. The validation of the proposed method was carried out as per ICH Q2B Guidelines [(I)]. It was found that the drug was shown the linearity between the range 2–20 µg/ml for each drugs respectively. The percentage recovery values of pure drug from the reanalyzed solution of formulation were in between 95.68% to 99.09% for lamivudine dextrose 97.56% to 99.45 for lamivudine xylose. The ruggedness of the method was studied by taking in account of varying the temperature. Based on the performance characteristic the proposed UV method was found to suitable for the estimation of lamivudine dextrose & lamivudine xylose.

Keywords: Lamivudine dextrose, Lamivudine xylose, UV.

INTRODUCTION

Lamivudine is 4-amino-1-[(2R,5S)-2-(hydroxylmethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination [(II)]. The drug is official in I.P and is indexed in other sources also [(III)]. A literature survey reveals the report of analytical methods for the determination of the drug individually and in combination with other drug in biological samples and in their dosage forms based on HPLC, HPTLC and LC-MS/MS. This report presents UV method development of Lamivudine dextrose & Lamivudine xylose.

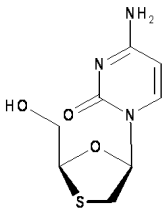

Fig. 1: Structure of Lamivudine

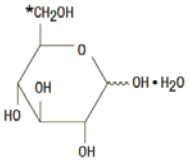

Fig. 2: Structure of Dextrose

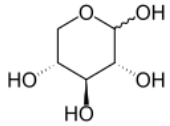

Fig. 3: Structure of Xylose

MATERIALS AND METHODS

Instrumentation

Simple UV Spectrophotometric Method was used. Lamivudine working standard was supplied by Cipla India Pvt. Ltd. Goa. All other chemicals used in the analysis were AR grade. A double – beam spectrophotometer (JASCO V-630) was used for the detection of absorbance, Electronic Balance (Shimadzu 200) and Sonicator (UCB 70) were used for experimental purpose. The 10 ml, 100 ml volumetric flasks, 100 ml measuring cylinder were used for preparation of standard & working solutions.

Preparation of stock solution

Standard stock solution was prepared by dissolving 10 mg of the drug in 100 ml of water to get the concentration of 100 µg/ml solution for each samples of lamivudine dextrose & lamivudine xylose. Then these stock solutions were heated in water bath for an hour. After heating dilutions were made in 10 ml volumetric flasks to get 2 to 20µg/ml for each drug respectively.

Preparation of working solution

From the above stock solution 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml & 2 ml were transferred into 10ml volumetric flasks and volume was make up to the mark with water to make 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml & 20 µg/ml respectively. Then the samples were scanned with UV-VIS Spectrophotometer against water as blank and the wavelength corresponding to maximum absorbance was noted which was its λ_{max}.

RESULTS

Determination of working wavelength (λ_{MAX})

In order to ascertain the wavelength of maximum absorption (λ max) of the drug, different solutions of the drugs (2µg/ml to 20µg/ml) in water was scanned using UV-VIS spectrophotometer within the wavelength region of 200 – 400 nm against water as blank. The resulting spectra were shown in figure no. 4 & 5 below and the absorption curve shows characteristic absorption maxima at 271nm for lamivudine dextrose & lamivudine xylose.

Preparation of calibration curve

Different concentrations from 2 to 20µg/ml were prepared from the stock solution in different volumetric flasks. Then the absorbance's were noted by scanning in UV. Then a calibration curve was plotted taking concentration on X-axis and absorbance on Y-axis. The calibration curve of lamivudine dextrose & lamivudine xylose was shown in fig. no. 6 & 7 respectively.

Validation

Validation can be defined as (ICH) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose. The validation for UV method development was performed using parameters like Linearity, Accuracy, Precision, Ruggedness, Limit of detection (LOD) & Limit of quantitation (LOQ) [1].

Linearity

Various aliquots were prepared form the stock solution (100µg/ml) ranging 2-20µg/ml. The samples were scanned in UV-VIS Spectrophotometer against water as blank. It was found that the selected drug shows linearity between the ranges of 2-20µg/ml. The results are shown in table no. 1 & 2 for lamivudine dextrose & lamivudine xylose respectively. The optical characteristics are as shown in table no. 3 & 4 respectively.

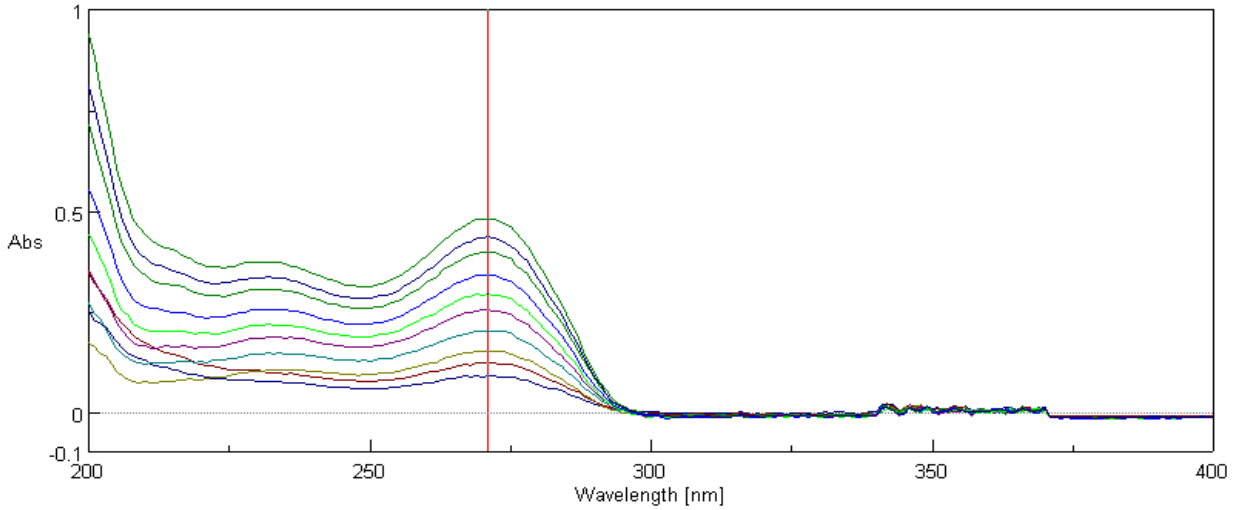

Fig. 4: UV – VIS Overlay spectrum of Lamivudine Dextrose for concentrations 2 – 20 µg/ml

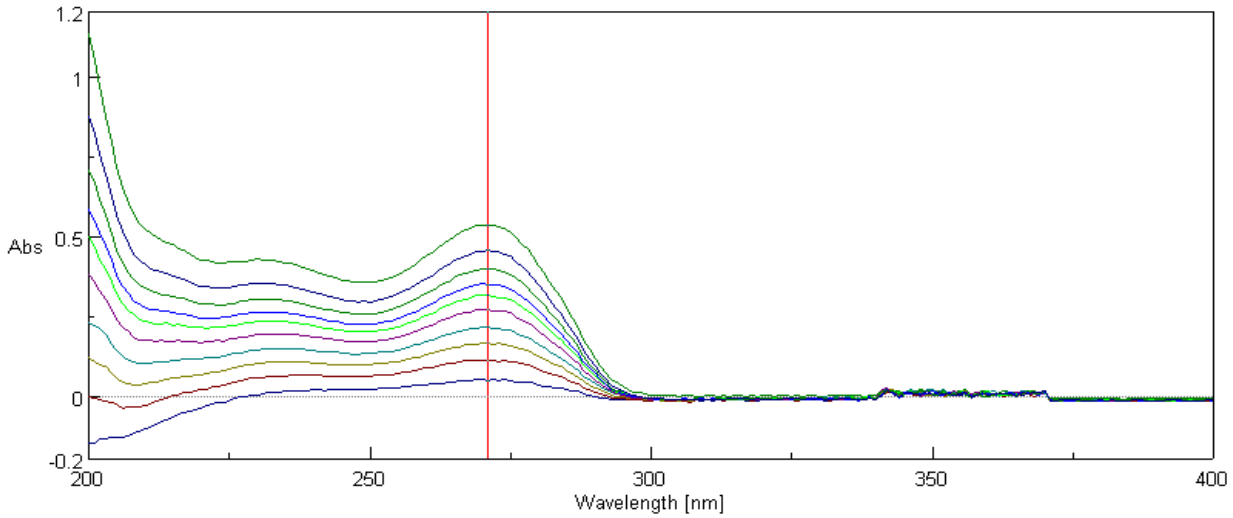

Fig. 5: UV – VIS Overlay spectrum of Lamivudine Xylose for concentrations 2 – 20 µg/ml

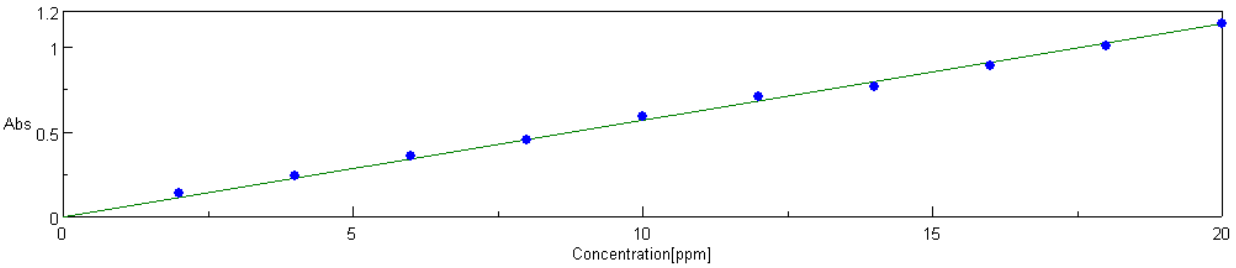

Fig. 6: Calibration curve of Lamivudine Dextrose for concentrations 2 – 20 µg/ml

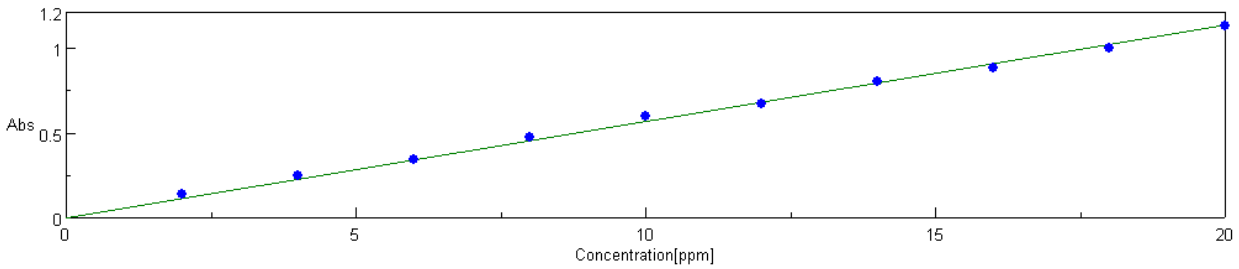

Fig. 7: Calibration curve of Lamivudine Xylose for concentrations 2 – 20 µg/ml

Table 1: Linearity Table of Lamivudine Dextrose

Serial No.	Concentration	Absorbance
1	2	0.1425
2	4	0.2456
3	6	0.3613
4	8	0.4536
5	10	0.5862
6	12	0.7025
7	14	0.7611
8	16	0.8888
9	18	1.0032
10	20	1.1282
	Correlation Coefficient	0.9990

Table 2: Linearity Table of Lamivudine Xylose

Serial No.	Concentration	Absorbance
1	2	0.1423
2	4	0.2483
3	6	0.3426
4	8	0.4754
5	10	0.5949
6	12	0.6707
7	14	0.7973
8	16	0.8762
9	18	0.9905
10	20	1.1256
	Correlation Coefficient	0.9990

Table 3: Optical Characteristics of Lamivudine Dextrose

Beer's law limit	2 – 20 micrograms/ml
Slope (a)	0.054093
Correlation coeff.	0.9990
Intercept (b)	0.032273
% Relative standard deviation	52.25
Regression equation (y*)	Y = 0.054093 X 0.032273
LOD	0.30 µg/ml
LOQ	0.60 µg/ml

Table 4: Optical Characteristics of Lamivudine Xylose

BEER'S LAW LIMIT	2 – 20 micrograms/ml
Slope (A)	0.053802
Correlation coeff.	0.9990
Intercept (B)	0.03456
% Relative standard deviation	52.06
Regression equation (Y*)	Y = 0.053802 X 0.03456
LOD	0.25 µg/ml
LOQ	0.75 µg/ml

Accuracy

Solutions in triplicate were prepared at levels 80%, 100% and 120% oftest concentration using Derivatives of Lamivudine and Nevirapine working standard as per the test method and absorbance of each solutions were taken. The recovery result showed that the proposed method has an acceptable level of accuracy for Lamivudine dextrose & Lamivudine xylose, which was between 97.63% to 99.45% for the test concentrations ranging from 80% to 120%. The accuracy readings are shown in table no. 5 & 6.

Table 5: Accuracy Reading for Lamivudine Dextrose

Sample	Concentration of drug (µg/ml)	Concentration of pure drug (µg/ml)	% Recovery of drug	Statistical Analysis
S1 : 80%	8	10	99.09	Mean = 98.95
S2 : 80%	8	10	99.05	SD = 0.2088
S3 : 80%	8	10	98.71	% RSD = 0.21
S4 : 100%	10	10	98.97	Mean = 98.60
S5 : 100%	10	10	97.63	SD = 0.8514
S6 : 100%	10	10	99.21	% RSD = 0.86
S7 : 120%	12	10	98.37	Mean = 97.29
S8 : 120%	12	10	95.68	SD = 1.4230
S9 : 120%	12	10	97.83	% RSD = 1.46

Table 6: Accuracy Reading for Lamivudine Xylose

Sample	Concentration of pure drug	Concentration of formulation	% Recovery of pure drug	Statistical Analysis
S1 : 80%	8	10	98.60	Mean = 98.60
S2 : 80%	8	10	99.44	SD = 0.8300
S3 : 80%	8	10	97.78	% RSD = 0.8417
S4 : 100%	10	10	99.45	Mean = 98.54
S5 : 100%	10	10	97.89	SD = 0.8087
S6 : 100%	10	10	98.30	% RSD = 0.8206
S7 : 120%	12	10	97.56	Mean = 98.42
S8 : 120%	12	10	99.15	SD = 0.8029
S9 : 120%	12	10	98.55	% RSD = 0.8157

Precision

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method.

From the absorbance results mean, Standard Deviation and % RSD were calculated and these were within the limit. The precision result showing repeatability of lamivudine dextrose & lamivudine xylose are shown in table no. 7 & 8. The intraday and interday precision results are given in table no. 9 to 12 respectively.

Table 7: Precision Result Showing Repeatability of Lamivudine Dextrose

S. No.	Concentration µg/ml	Absorbance	
1.	10	0.5862	Mean = 0.5815
2.	10	0.5876	SD = 0.006929
3.	10	0.5767	%RSD = 1.1915
4.	10	0.5678	
5.	10	0.5780	
6.	10	0.5878	
7.	10	0.5844	
8.	10	0.5839	

Table 8: Precision Result Showing Repeatability of Lamivudine Xylose

S. No.	Concentration µg/ml	Absorbance	
1.	8	0.4754	Mean = 0.4736
2.	8	0.4679	SD = 0.005191
3.	8	0.4768	%RSD = 1.0960
4.	8	0.4788	
5.	8	0.4769	
6.	8	0.4786	
7.	8	0.4677	
8.	8	0.4669	

Table 9: Intraday Precision Study of Lamivudine Dextrose

Concentration µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Average %RSD
8	0.4536	0.4623	0.4576	1.3095
8	0.4590	0.4588	0.4529	
8	0.4678	0.4512	0.4501	
8	0.4486	0.4528	0.4689	
8	0.4549	0.4502	0.4569	
8	0.4532	0.4579	0.4599	
%RSD	1.4463	1.0608	1.4216	

Table 10: Intraday Precision Study of Lamivudine Xylose

Concentration µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Average %RSD
10	0.5949	0.5978	0.5801	0.6256
10	0.5909	0.5967	0.5958	
10	0.5918	0.5938	0.5943	
10	0.5934	0.5998	0.5967	
10	0.5988	0.5992	0.5944	
10	0.5928	0.5986	0.5927	
%RSD	0.4749	0.3641	1.0379	

Table 11: Interday Precision Study of Lamivudine Dextrose

Concentration µg/ml	Day 1	Day 2	Day 3	Average % RSD
12	0.7025	0.7035	0.7056	0.4100
12	0.7055	0.7098	0.7088	
12	0.7034	0.7031	0.7025	
12	0.7078	0.7087	0.7086	
12	0.7012	0.7067	0.7001	
12	0.7033	0.7079	0.7077	
% RSD	0.3338	0.3911	0.5051	

Table 12: Interday Precision Study of Lamivudine Xylose

Concentration µg/ml	Day 1	Day 2	Day 3	Average % RSD
12	0.6707	0.6774	0.6736	0.4741
12	0.6788	0.6752	0.6786	
12	0.6723	0.6735	0.6755	
12	0.6798	0.6725	0.6727	
12	0.6755	0.6712	0.6793	
12	0.6744	0.6767	0.6699	
% RSD	0.5278	0.3607	0.5340	

Ruggedness

For the ruggedness testing parameters selected were room temperature & 20^{o}C**.** The results are given in table no. 13 to 16 respectively.

Table 13: Ruggedness Results at Room Teperature for Lamivudine Dextrose

Concentration µg/ml	Absorbance	Statistical Analysis
10	0.5862	Mean = 0.5733
10	0.5860	SD = 0.010039
10	0.5658	% RSD = 1.7510
10	0.5645	
10	0.5678	
10	0.5698	

Table 14: Ruggedness Results at Room Teperature for Lamivudine Xylose

Concentration µg/ml	Absorbance	Statistical Analysis
12	0.6707	Mean = 0.6750
12	0.6712	SD = 0.003382
12	0.6789	% RSD = 0.5010
12	0.6776	
12	0.6756	
12	0.6764	

Table 15: Ruggedness Results at Teperature 20^{o}c for Lamivudine Dextrose

Concentration µg/ml	Absorbance	Statistical Analysis
12	0.7025	Mean = 0.7051
12	0.7033	SD = 0.002654
12	0.7045	% RSD = 0.3764
12	0.7065	
12	0.7098	
12	0.7042	

Table 16: Ruggedness Results at Teperature 20°C for Lamivudine Xylose

Concentration µg/ml	Absorbance	Statistical Analysis
10	0.5949	Mean = 0.5943
10	0.5955	SD = 0.002456
10	0.5934	% RSD = 0.4132
10	0.5929	
10	0.5912	
10	0.5983	

Limit of Detection (LOD)

The LOD for lamivudine dextrose was found to be 0.30 µg/ml & lamivudine xylose was found to be 0.25 µg/ml.

LIMIT of Quantitation (LOQ)

The LOQ for lamivudine dextrose was found to be 0.60 µg/ml & lamivudine xylose was found to be 0.75 µg/ml.

DISCUSSION

The developed spectrophotometric method was simple, sensitive, specific and reliable with good precision and accuracy. The value of correlation coefficient was 0.999 which shows the sensitivity of the method. Hence, this method can be used for routine determination of lamivudine dextrose & lamivudine xylose in bulk.

CONCLUSIONS

The developed spectrophotometric method was simple, sensitive, specific and reliable with good precision and accuracy. The precision results showing the higher value for %RSD was 1.13 which was within limits. The higher limit for %RSD was 2%, hence this method fit the precision limits. Hence, this method can be used for routine determination of Derivatives of lamivudine dextrose, lamivudine xylose in bulk.

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