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DEVELOPMENT AND VALIDATION OF NEW UV SPECTROSCOPIC METHODS FOR THE ESTIMATION OF LAMIVUDINE IN ACTIVE PHARMACEUTICAL INGREDIENT AND IN ITS TABLET FORMULATION

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ABSTRACT

Lamivudine is an FDA approved drug for clinical use for the treatment of HIV infection, AIDS and AIDS-related conditions either alone or in combination with other antiviral agents. In this research work few rapid, simple, accurate and economical UV spectrophotometric methods have been developed and validated for the estimation of the anti-retroviral agent lamivudine in active pharmaceutical ingredient and in its tablet formulation. The λ_{max} of the proposed solvent blends were found to be at 272nm, 282nm, 272nm and shows linearity over the concentration range of 1-20µg/ml with a correlation coefficient of 0.9999, 0.9998, and 0.9997 for three solvent blends viz., Methanol: Double distilled water (3:1); Methanol: Double distilled water: 0.1N HCl (3:1:1); Methanol: Double distilled water: 0.1N NaOH (3:1:1) respectively. All the proposed methods were statistically validated for accuracy, precision, linearity, robustness, and ruggedness as per ICH guidelines. The % RSD values for validated methods were found to less than 2. These methods can be applied for the routine quantitation of lamivudine in API and its tablet formulation.

Keywords *– Lamivudine, UV-spectrophotometry, Double distilled water, 0.1N NaOH, 0.1N HCl*

1. INTRODUCTION

Analysis is an integral component of preformulation and formulation development research. It is essential to have a validated, stability indication/specific method of analysis for the drug for which delivery system is to be designed. UV spectrophotometer technique is one of the earliest and most widely applied detection techniques for drug estimation. UV spectrophotometer method is preferred over other technique for routine analysis as it is less time consuming and also cost effective. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results and it is an integral part of any good analytical practice. Lamivudine is a synthetic nucleoside analogue with activity against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infection[1,2]. It is phosphorylated intracellularly and inhibits HIV reverse transcriptase as well as hepatitis B virus (HBV) DNA polymerase. Its incorporation into DNA results in chain termination. Chemically lamivudine is a (2R, 5S)-4-amino-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5yl]-2(1H)-pyrimidinone[3,4], soluble in water, sparingly soluble in methanol[5,6]

and its chemical structure is shown in figure 1. The spectroscopic method for assay of lamivudine is not official in any pharmacopoeia. Literature survey revealed few UV spectrophotometer methods were reported for the estimation of lamivudine alone and in combination with other drugs in bulk and its formulations[7-13]. A few RP-HPLC[14-17], HPLC[18] techniques have been suggested for analysis of the lamivudine alone and in combination with other antiretroviral drugs. HPLC is the most widely used technique for the estimation of lamivudine in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The suggested RP-HPLC and HPLC methods for assay of lamivudine are expensive and need complex and sophisticated instrumentation. A first derivative of the ratio-spectra and high-performance liquid chromatography[19, 20], Titrimetric[21] methods are also reported for the estimation of lamivudine. Hence in the present work it was aimed to develop and validate accurate, precise, simple and rapid UV spectroscopic methods for the estimation of lamivudine in API and its Tablet formulations as per ICH guidelines.

2. MATERIALS AND METHODS

2.1 Materials

Lamivudine gift sample was obtained from Strides Arco lab, Bengaluru. Lamvir 100mg tablets were procured. Methanol, Hydrochloric acid and Sodium hydroxide were procured from S.D Fine chemicals Mumbai, double distilled water was used throughout the experiments.

2.2 Methods

2.2.1. Preparation of lamivudine standard stock solution (1000µg/ml)

Weighed accurately about 100 mg of lamivudine API and transferred in to a 100 ml volumetric flask to this add 50ml of Methanol: Double distilled water (3:1) (Method A) and sonicated for about 5 minutes to dissolve it and made to volume. Similarly prepare standard stock solutions in Methanol: Double distilled water: 0.1N HCl (3:1:1) (Method B); Methanol: Double distilled water: 0.1N NaOH (3:1:1) (Method C) solvent blends.

2.2.2. Determination of absorption maxima (λ max)

Appropriate aliquots from standard lamivudine stock solutions were transferred in to series of 10 ml volumetric flasks. The volume was adjusted to the mark with Methanol: Double distilled water (3:1) to get desired concentration. The obtained solutions were subjected for UV scanning in the range of 200-380 nm using double beam UV Spectrophotometer and determine the absorption maxima (λ max). Similarly determine the absorption maxima (λ max) of lamivudine in other two solvent blends viz., Methanol: Double distilled water: 0.1N HCl (3:1:1) and Methanol: Double distilled water: 0.1N NaOH (3:1:1).

2.2.3 Determination of linearity range

Appropriate aliquots from standard lamivudine stock solutions were transferred to series of 10 ml volumetric flasks. The volume was adjusted to the mark with Methanol: Double distilled water (3:1) to get desired concentration viz., 2-30µg/ml and determine the linearity range by measure the absorbance at 272nm taking the Methanol: Double distilled water (3:1) as the blank. Similarly determine the linearity range by measure the absorbance at 282nm and 272nm for Methanol: Double distilled water: 0.1N HCl (3:1:1) and Methanol: Double distilled water: 0.1N NaOH (3:1:1) solvent blend respectively.

2.2.4 Determination of calibration curve

Appropriate aliquots from standard lamivudine stock solutions were transferred in to series of 10 ml volumetric flasks. The volume was adjusted to the mark with Methanol: Double distilled water (3:1) to obtain concentrations of 1, 2, 4, 6, 8 and 10µg/ml and measure the absorbance at 272nm. Similarly prepare 1, 2, 4, 6, 8 and 10µg/ml concentration solution in Methanol: Double distilled water: 0.1N HCl (3:1:1) and Methanol: Double distilled water: 0.1N NaOH (3:1:1) solvent blends, measure the absorbance

at 282nm and 272nm. The concentration vs absorbance values were plotted and interpreted statistically.

2.3 Validation

2.3.1 Preparation of lamivudine sample solution (for tablets)

Ten lamivudine marketed tablets were procured, weighed and crushed uniformly in a glass mortar. An accurately weighed powder sample equivalent to 100 mg of lamivudine was transferred into a 100ml volumetric flask containing 50ml of Methanol: Double distilled water (3:1) and the contents were sonicated for about 5 min to enhance the dissolution and is completed in 15 min. Transfer aliquots through 0.45 µm membrane filter into 100ml volumetric flask and made the volume with Methanol: Double distilled water (3:1) solvent blend. Similarly, sample solutions were prepared in Methanol: Double distilled water: 0.1N HCl (3:1:1) and Methanol: Double distilled water: 0.1N NaOH (3:1:1). These sample solutions were further used for the validation studies.

2.3.2 Accuracy

The accuracy was evaluated applying the proposed methods to the analysis formulations with known amounts of drug. The studies were carried out in triplicate by adding known amount of standard drug (50% and 20%) to the sample solution measure the absorbance and calculate the amount of lamivudine recovered from the calibration curve. The accuracy was calculated as the percentage of the drug recovered from the formulations in terms of % RSD and it should be less than 2%.

2.3.3 Precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter day). Repeatability was evaluated assaying three determinations at the same concentration (10µg/ml), during the same day, under the same experimental conditions. Intermediate precision was analyzed comparing the assays in three determinations at the same concentration (10µg/ml) during 3 different days. Precision (repeatability and intermediate precision) was expressed as relative standard deviation (RSD). Intraday precision was determined by analyzing lamivudine content for three times in the same day (morning, afternoon, evening) by measuring the absorbance at 272nm in Method A and 282nm and 272nm in Method B and Method C respectively. Interday precision was determined by analyzing daily once (morning) for three days by measuring the absorbance at 272nm in Method A and 282nm and 272nm in Method B and Method C respectively. The % RSD values were calculated and it should be less than 2%.

2.3.4 LOD and LOQ

These parameters are not a requirement for drug assay, however it is always useful to demonstrate that the analyses are being conducted in a region which is above the LOQ value. The LOD and LOQ were calculated based on the standard deviation of the response (y-intercepts of regression lines) and the slope using three independent analytical curves, as denied by ICH. The lowest possible concentration where the drug lamivudine show response was determined in the three methods viz., Method A, Method B and Method C. The absorbance at this concentration was measured in triplicate in Method A, Method B and Method C at 272nm; 282nm; 282nm respectively. The LOD/LOQ was calculated by using following formulae from the data obtained.

$$\text{LOD (µg/ml)} = 3.3 \times \frac{\sigma}{s} \qquad \text{LOQ (µg/ml)} = 10 \times \frac{\sigma}{s}$$

Where σ - Standard deviation of the response; s – Slope ratio curve

2.3.5 Robustness

Robustness of the proposed methods were determined by the analysis of samples and standard solutions (10µg/ml) at different wavelengths (±5nm), at different solution temperatures (refrigeration condition 2-8 ºC and 37ºC). To assess the stability of drug, the stability study was performed maintaining the drug working solution in respective solvent systems for 48h protected from light, looking for the decrease of absorbance compared with those of freshly prepared solutions. Appropriate concentrations of

lamivudine from API and its tablet formulations were prepared in three methods viz., Method A, Method B and Method C. Amount found was calculated at three different wavelengths (actual and ± 5 nm) in terms of % RSD and values should be less than 2%.

2.3.6 Ruggedness

Ruggedness is not addressed in the ICH documents. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from analyst to analyst and instrument to instrument. Appropriate concentrations of lamivudine from bulk and formulations were prepared in Method A, Method B and Method C. Analysis was carried out by two different analysts and also two instruments. Amount found was in terms of % RSD and values should be less than 2%.

3. RESULTS AND DISCUSSION

Simple, rapid, economic, accurate, precise and sensitive UV spectrophotometric methods were developed and validated as per ICH guideline and USP 2000 for the estimation of lamivudine in API and its tablet formulations (Lamvir 100mg). Three methods viz., Method A, Method B and Method C were selected. The developed methods were further validated for accuracy, precision, LOD, LOQ, specificity, robustness, and ruggedness with statistical data. The absorption maxima (λmax) with characteristic peak for lamivudine were found at 272nm, 282nm and 272nm for in Method A, Method B and Method C respectively. These absorption maxima were used to determine the linearity and it was shown linear relationship with correlation coefficient of 0.9999; 0.9998 and 0.9997 for in Method A, Method B and Method C respectively in the concentration range of 1-10 µg/ml. The spectra and data were shown in figure 2, 3 and table1. The calibration curve for lamivudine in Method A, Method B and Method C were prepared in the concentration range of 1-10 µg/ml. In all the methods the P value is < 0.0001 indicate proposed methods were found to be statistically significant. The calibration curve data and statistical data were shown in table 2, 3 and calibration curve in figure 4. The percentage recovery of the drug was found to be in the range of 99-100%; 99.4%-100.5%; 99.4%-100.5% in Method A, Method B and Method C respectively for the estimation of lamivudine in API. The percent recovery at each level was found to be well within the range, indicating insignificant interference from the excipients. The data were given in table 4. The % recovery of lamivudine was found to be satisfactory with % RSD values are 0.824, 0.902; 0.804, 0.915; 0.691 0.830 for Method A, Method B and Method C respectively which were within the acceptance limit. The results suggest that proposed methods were accurate in estimation. The data were shown in table 5. Based on the standard deviation of the response and the slope the limit of detection values for lamivudine were found to be 0.182 µg/ml, 0.381 µg/ml, 0.412 µg/ml and limit of quantitation were found to be 1.084 µg/ml, 1.36 µg/ml, 1.054 µg/ml for Method A, Method B and Method C respectively. The data were shown in table 6. The % RSD values of intraday and inter day precision for capecitabine in formulations were found to be less than 1.5 for Method A, Method B and Method C respectively which were within the acceptance limit. The results suggest the proposed methods were precise and reproducible for the estimation. The data was shown in table 7. Change in the λmax of ± 5nm to the actual λmax in robust analysis the % recovery of lamivudine was found to be significantly different which clearly indicates change in λmax of 5nm affected the method so proposed methods were not robust. Similarly change in the storage conditions during robust analysis, the % recovery lamivudine is found to be significantly different which clearly indicates the storage condition is also affecting the method so proposed methods were not robust. The robust data were given in table 8, 9. The % recovery of capecitabine in ruggedness analysis by different analyst and change of instrument viz., analyst-1; analyst-2 and instrument-1; instrument-2 shows the proposed methods were significantly rugged. The ruggedness data were shown in table 10, 11.

4. CONCLUSION

The proposed UV spectrophotometric methods were found to be simple, rapid, accurate, precise and economic. From the above data it was observed that all validation parameters meet the predetermined acceptance criteria and validated in terms of linearity, accuracy, precision, reproducibility, robustness, and ruggedness as per the ICH guidelines. Thus, it has been concluded that the proposed methods were validated for the analysis of lamivudine in API and its tablet formulations.

Table 1: Linearity range data of lamivudine in Method A, Method B and Method C.

Conc. (µg/ml)	Method A	Method B	Method C
	Absorbance* ± SD	**Absorbance*** ± SD	**Absorbance*** ± SD
2	0.088±0.004	0.089±0.009	0.091±0.002
4	0.172±0.007	0.182±0.008	0.180±0.005
6	0.262±0.002	0.271±0.003	0.269±0.009
8	0.352±0.009	0.352±0.006	0.361±0.005
10	0.444±0.007	0.438±0.008	0.448±0.007
12	0.519±0.008	0.528±0.002	0.544±0.008
18	0.784±0.008	0.766±0.005	0.773±0.002
22	0.887±0.002	0.891±0.006	0.875±0.008
24	0.912±0.003	0.921±0.008	0.931±0.006
28	0.998±0.001	0.979±0.005	0.991±0.004
30	1.012±0.002	1.021±0.003	1.009±0.008

Table 2: Calibration curve data of lamivudine in Method A, Method B and Method C

Conc. (µg/ml)	Method A **Absorbance*** ± SD	Method B **Absorbance*** ± SD	Method C **Absorbance*** ± SD
0	0.0000	0.0000	0.0000
1	0.045 ± 0.0063	0.074 ± 0.0022	0.047 ± 0.0061
2	0.090 ± 0.0072	0.144 ± 0.0100	0.092 ± 0.0070
4	0.178 ± 0.0076	0.276 ± 0.0049	0.180 ± 0.0052
6	0.272 ± 0.0065	0.407 ± 0.0174	0.272 ± 0.0146
8	0.362 ± 0.0065	0.548 ± 0.0191	0.360 ± 0.0050
10	0.456 ± 0.0017	0.683 ± 0.0038	0.450 ± 0.0079

**Average of six determinations*

Table 3: Statistical data of calibration curve for lamivudine in Method A, Method B and Method C.

Parameters	Method A	Method B	Method C
λ_{max}(nm)	272	282	272
Beer's law limits (µg / ml)	1-12	1-12	1-12
Molar Absorptivity ($mol^{-1}cm^{-1}$)	10.4×10^3	15.5×10^3	10.4×10^3
Sandell's sensitivity	0.022	0.014	0.022
Best-fit values			
Slope	0.04553 ± 0.000190	0.06785 ± 0.0003720	0.04490 ± 0.0001195
Y-intercept when X=0.0	-0.001215 ± 0.00106	0.004078 ± 0.002090	0.001311 ± 0.0006716
X-intercept when Y=0.0	0.02668	-0.06011	-0.02919
1/slope	21.96	14.74	22.27
95% CI			
Slope	0.04504 to 0.04602	0.06690 to 0.06881	0.04459 to 0.04520
Y-intercept when X=0.0	-0.003961 to 0.00153	-0.001295 to 0.00945	-0.0004160 to 0.00303
X-intercept when Y=0.0	-0.03392 to 0.0862	-0.1409 to 0.01887	-0.06802 to 0.009217
Goodness of Fit			
R square	0.9999	0.9998	0.9997
P value	< 0.0001	< 0.0001	< 0.0001

Table 4: Percentage recovery data of lamivudine in proposed methods

METHOD A			
Sample No	**Concentration of lamivudine (µg/ml)**		**% Recovery***
	Theoretical	Experimental	
1	2	1.98	99±0.112
2	4	3.97	99.2±0.100
3	6	6	100±0.093
4	8	8.0	100±0.125
5	10	9.99	99.9±0.141
METHOD B			
	Theoretical	Experimental	
1	2	2.01	100.5±0.124
2	4	4.02	100.5±0.101
3	6	6	100±0.156
4	8	7.94	99.4±0.112
5	10	10.1	100.1±0.117
METHOD C			
	Theoretical	Experimental	
1	2	2.0	100±0.122
2	4	3.9	99.4±0.114
3	6	6.03	100.5±0.125
4	8	8	100±0.151
5	10	10.1	100.1±0.146

**Average of six determinations*

Table 5: Data showing recovery studies of lamivudine (formulations) in proposed methods.

METHOD A					
Amount present in formulation (µg/ml)	**Amount added**		**Amount recovered (µg/ml)**	**Mean % Recovery ± SD**	**RSD**
	µg	**%**			
10	5	50	14.95	99.0 ± 1.259	0.824
	2	20	11.98	99.0 ± 1.629	0.902
METHOD B					
Amount present in formulation (µg/ml)	**Amount added**		**Amount recovered (µg/ml)**	**Mean % Recovery ± SD**	**RSD**
	µg	**%**			
10	5	50	14.99	99.90 ± 1.908	0.804
	2	20	12.03	100.3 ± 1.219	0.915
METHOD C					
Amount present in formulation (µg/ml)	**Amount added**		**Amount recovered (µg/ml)**	**Mean % Recovery ± SD**	**RSD**
	µg	**%**			
10	5	50	14.96	99.20 ± 1.707	0.691
	2	20	12.	100.0 ± 1.143	0.830

Table 6: Data showing LOD/LOQ of lamivudine in proposed methods.

METHOD A		
	Mean ± SD	**SEM**
Limit of detection	0.182 ± 0.046	0.022
Limit of quantitation	1.084 ± 0.145	0.081
METHOD B		
	Mean ± SD	**SEM**
Limit of detection	0.381 ± 0.047	0.037
Limit of quantitation	1.36 ± 0.148	0.074
METHOD C		
	Mean ± SD	**SEM**
Limit of detection	0.412 ± 0.134	0.082
Limit of quantitation	1.054 ± 0.384	0.211

Table 7: Data showing precision Intraday and Inter day trials with RSD values for lamivudine in proposed methods.

METHOD A							
Trials	**Label claim (mg/tab)**	**Amount found (mg/tab)**		**% Label claim Mean ± SD**		**SEM**	**RSD**
Day-1	50	50.6 50.3 49.7		101.2± 1.212 100.6 ± 0.651 99.4 ± 0.512		0.629 0.412 0.353	1.219 0.741 0.554
Day-2	50	50.2 49.5 49.8	**Intra day trials**	100.4 ± 1.371 98.4 ± 1.162 99.2 ± 0.982	**Intra day trials**	0.751 0.611 0.812	1.271 1.023 1.138
Day-3	50	50.6 50.04 49.9		101.2 ± 0.791 100.04 ± 0.862 99.8 ± 0.513		0.300 0.100 0.372	0.612 0.912 0.512
METHOD B							
Day-1	50	50.4 50.4 50.3		100.8 ± 1.182 100.8 ± 0.815 100.6 ± 1.014		0.801 0.441 0.592	1.221 0.758 1.016
Day-2	50	50.6 50.4 50.0	**Intra day trials**	101.2 ± 0.562 100.8 ± 1.104 100.0 ± 0.470	**Intra day trials**	0.374 0.612 0.493	0.612 1.096 0.698
Day-3	50	50.4 49.9 49.7		100.8 ± 0.101 99.8 ± 0.986 99.4 ± 0.888		0.215 0.710 0.614	0.189 1.088 0.891
METHOD C							
Day-1	50	49.9 49.9 49.8		99.8 ± 0.712 99.8 ± 0.891 99.6 ± 0.912		0.516 0.658 0.758	0.712 0.786 0.903
Day-2	50	50.3 50.0 49.9	**Intra day trials**	100.6± 1.005 100.0 ± 1.023 99.81 ± 0.917	**Intra day trials**	0.715 0.913 0.412	1.056 1.125 0.746
Day-3	50	50.6 49.9 49.9		101.2 ± 1.22 99.8 ± 0.756 99.8 ± 1.151		0.805 0.412 0.671	1.013 0.734 1.016

Table 8: Data showing robustness of lamivudine at different wavelengths in proposed methods

METHOD	Conc (⍰g/ml)	Wave length	Amount found	Mean % ± SD	SEM	RSD
METHOD A	10	272	9.98	99.8 ± 0.862	0.452	0.915
		277	8.27	82.7 ± 1.615	0.912	1.417
		267	8.41	84.1 ± 1.021	0.711	1.213
METHOD B	10	282	9.97	99.7 ± 0.612	0.517	0.712
		287	8.32	83.2 ± 1.007	0.912	1.520
		277	8.5	85 ± 1.059	0.612	1.311
METHOD C	10	272	9.99	99.9 ± 1.241	0.721	1.256
		277	8.41	84.1 ± 1.112	0.802	1.112
		267	8.52	85.2 ± 0.978	0.662	1.126

Table 9: Data showing Robustness of lamivudine at refrigerated condition and room temperature in proposed methods.

	Trials	Label Claim (mg/tab)	REFREGERATED CONDITION						ROOM TEMPERATURE					
			Amount Found (mg/tab)		% Label Claim Mean ± SD		SEM	RSD	Amount Found (mg/tab)		% Label Claim Mean ± SD		SEM	RSD
Method-A	Day-1	50	48.5 49.0 48.8	Intra day trials	97 ± 0.321 98 ± 0.250 97.6 ± 0.232	Intra day trials	0.211 0.218 0.212	0.332 0.342 0.345	50.2 50.2 49.9	Intra day trials	100.4 ± 1.149 100.4 ± 0.823 99.8± 0.632	Intra day trials	0.627 0.324 0.313	1.133 0.823 0.667
	Day-2	50	48.8 48.6 48.5		97.6 ± 0.251 97.2± 0.241 97.0 ± 0.211		0.223 0.156 0.162	0.250 0.351 0.453	50.04 49.7 49.8		100.08 ± 1.274 99.4 ± 1.161 99.6 ± 1.183		0.672 0.721 0.910	1.374 1.011 1.212
	Day-3	50	48.6 49.1 49.2		97.2 ± 0.413 98.2 ± 0.222 98.4 ± 0.212		0.288 0.145 0.161	0.522 0.253 0.292	50.2 50.01 49.9		100.4 ± 0.792 100.02 ± 0.86 99.8 ± 0.456		0.300 0.101 0.173	0.621 0.934 0.513
Method-B	Day-1	50	48.9 49.0 49.1	Intra day trials	97.8 ± 0.503 98 ± 0.421 98.2 ± 0.120	Intra day trials	0.128 0.220 0.188	0.500 0.349 0.371	50.2 50.2 50.1	Intra day trials	100.4 ± 1.287 100.4 ± 0.863 100.2 ± 1.126	Intra day trials	0.700 0.341 0.692	1.278 0.858 1.116
	Day-2	50	49.0 48.8 48.8		98.0 ± 0.451 97.6 ± 0.351 97.6 ± 0.321		0.412 0.320 0.246	0.250 0.421 0.398	50.2 50.0 50.0		100.4 ± 0.565 100.0 ± 1.101 100.0 ± 0.981		0.234 0.673 0.433	0.718 1.006 0.988
	Day-3	50	48.6 48.9 49.0		97.6 ± 0.350 97.8 ± 0.416 98 ± 0.230		0.210 0.421 0.312	0.333 0.420 0.413	50.1 49.9 49.8		100.2 ± 0.400 99.8 ± 1.186 99.6 ± 0.865		0.134 0.858 0.614	0.122 1.318 0.973
Method-C	Day-1	50	48.9 48..8 48.6	Intra day trials	97.8 ± 0.230 97.6± 0.550 97.2 ± 0.472	Intra day trials	0.213 0.118 0.132	0.319 0.534 0.421	50.1 49.9 49.9	Intra day trials	100.2 ± 0.622 99.8 ± 0.894 99.8 ± 0.723	Intra day trials	0.516 0.328 0.568	0.818 0.732 0.914
	Day-2	50	48.9 48.6 49.0		97.8 ± 0.423 97.2 ± 0.401 98.0 ± 0.411		0.222 0.310 0.464	0.374 0.4070.512	50.3 50.1 49.9		100.6± 1.233 100.2 ± 1.165 99.8 ± 0.511		0.765 0.933 0.888	1.056 0.975 1.321
	Day-3	50	49 48.9 48.8		98 ± 0.321 97.8 ± 0.612 97.6 ± 0.346		0.165 0.278 0.224	0.313 0.561 0.422	50.3 49.8 49.9		100.6 ± 1.121 99.6 ± 0.657 99.8 ± 1.220		0.805 0.217 0.564	1.012 0.954 1.216

Table 10: Data showing ruggedness of lamivudine by different Analysts in proposed methods

METHOD	Conc (µg/ml)	Analyst	Amount found	Recovery ± SD	SEM	RSD
METHOD A	10	Analyst 1	10.00	100.0± 0.451	0.123	0.129
		Analyst 2	9.98	99.8 ± 0.312	0.212	0.401
METHOD B	10	Analyst 1	9.99	99.9 ± 0.721	0.217	0.816
		Analyst 2	10.02	100 .4± 0.345	0.111	0.592
METHOD C	10	Analyst 1	10.02	100.2 ± 0.213	0.123	0.423
		Analyst 2	9.99	99.9 ± 0.343	0.167	0.512

Table 11: Data showing ruggedness of lamivudine by using different Instruments in proposed methods

METHOD	Conc (µg/ml)	Instrument	Amount found	Recovery ± SD	SEM	RSD
METHOD A	10	Instrument 1	9.97	99.7 ± 0.863	0.254	0.864
		Instrument 2	10.03	100.3 ±0.243	0.212	0.592
METHOD B	10	Instrument 1	9.99	99.9± 0.709	0.409	0.733
		Instrument 2	9.91	99.1 ± 0.642	0.371	0.658
METHOD C	10	Instrument 1	9.92	99.2 ± 0.949	0.621	1.051
		Instrument 2	9.93	99.3 ± 0.520	0.626	0.820

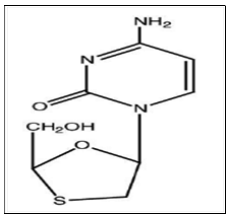

Figure 1: Chemical structure of Lamivudine.

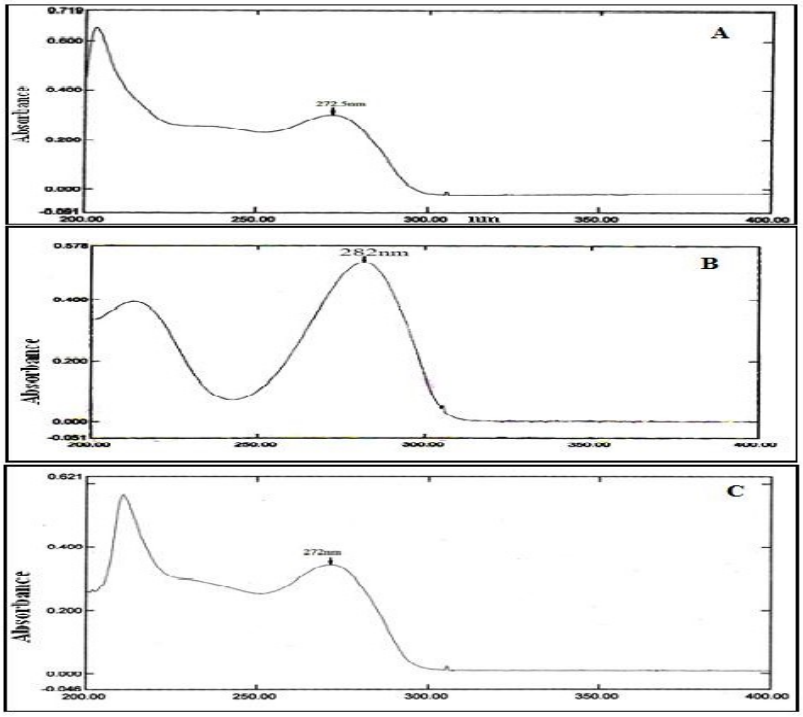

Figure 2: Absorption maxima of lamivudine in Method A, Method B and Method C

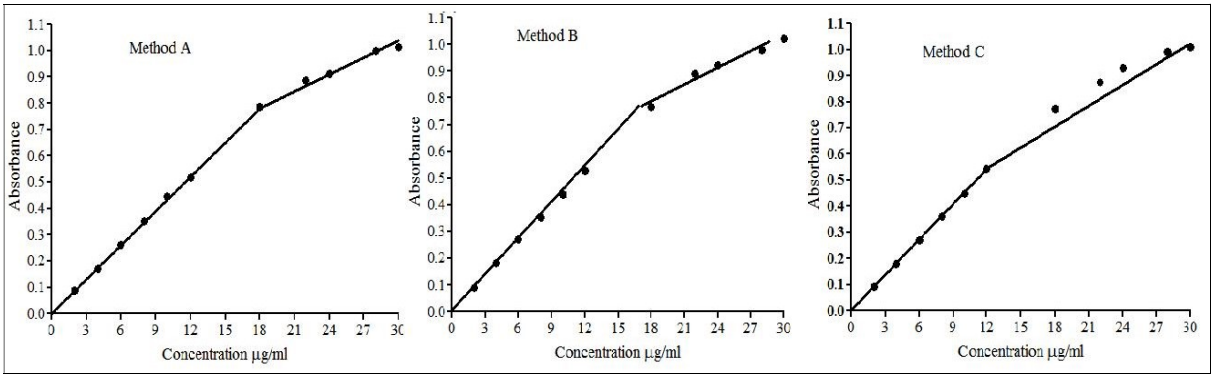

Figure 3: Linearity range curve of lamivudine in Method A, Method B and Method C

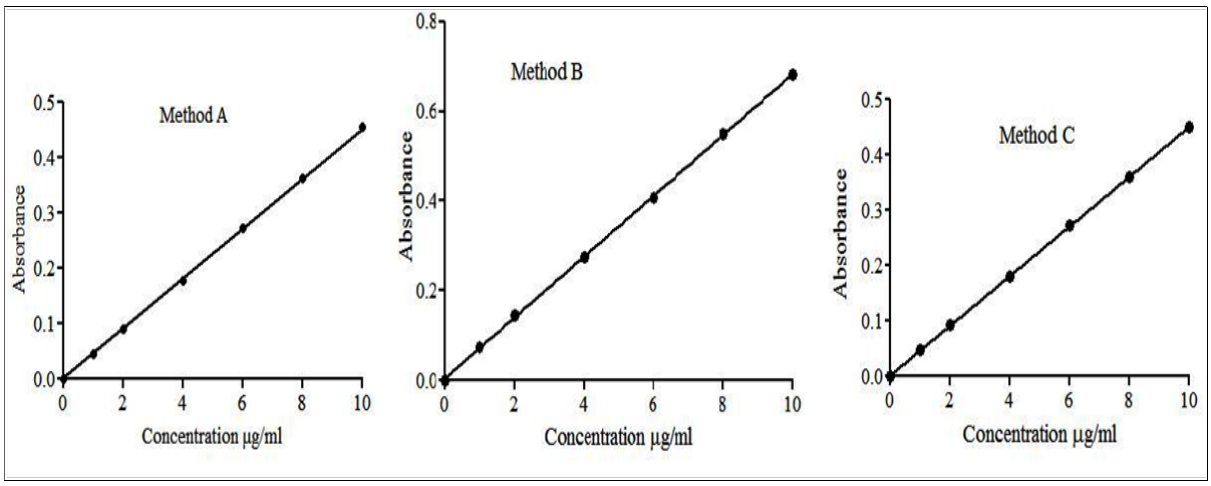

Figure 4: Calibration curve of lamivudine in Method A, Method B and Method C

5. ACKNOWLEDGEMENT

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