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Development and Validation of UV Spectroscopic Method for Estimation of Lamivudine in Tablet Dosage Form

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ABSTRACT:- It is simple, rapid sensitive precise and specific UV spectrophotometric method for the determination of lamivudine in bulk drug and tablet dosage form were developed and validated. A simple double beam UV spectroptometric method has been developed and validated with different parameter such as linearity, precision .The drug freely soluble in DMF. The drug was identified in term of solubility studies and on the basis of melting point done on melting point apparatus of equiptronics. Itshowed absorption maxima were determined in DMF. The drug obeyed the beer's law and sowed a good correlation of concentration with absorption which reflex in linearity .The UV spectroscopic method was developed for estimation of lamivudine in tablet dosage form and also validated as per ICH guidelines. The drug is freely in DMF slightly soluble in methanol and practically insoluble in acetone so the DMF is used as a diluentin the method. The melting point of lamivudine was found to be 160- 161^0c . It showed absorption maxima 268 nm is an DMF . On the basis of the absorption spectrum, the working concentration was set on 10µg/ml (PPM). The linearity was observed between 10-50 µg/ml. The result of the analysis were validated by recovery studies. The recovery was found to be 99.2% for three level respectively.
Keywords:- DMF, UV spectroscopy, linearity, precision, accuracy.

INTRODUCTION

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI). It is a phosphorylated intracellular to its active from lamivudine- 5'- triphosphate by the successive action of deoxycytidine kinas. cytidine monophosphate kinase

. It reduce HIV-1 p24 antigen levels in the cultural supernatant of human peripheral blood lymphocytes infected with laboratory adopted HIV-1 strain, indicating inhibition of HIV replication. Lamivudine inhibits syncytium formation between various $CD4^+$cell lines and several strains of HIV-1 . It reduce serum viral titters in a woodchuck model of chronic hepatitis B infection when administered at dose of 40 or 200 mg/kg. formulation containing lamivudine have been used in the treatment of HIV and HBV infection. Reverse transcriptase inhibitor like lamivudine have been used in combination o treat patients with HIV. The treatment is used to prevent or prolong the onset of (AIDS) acquired immune deficiency syndrome. Which can lead to a variety of fatal complication. Lamivudine is also used in lower doses to treat patient with chronic hepatitis B in whom the virus has replicated and caused liver inflammation.

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Literature review, it's found that one method was reported on derivative spectrophotometer for simultaneous estimation of lamivudine their combined dosage form Also, the method was reported on human serum and drug.

UV method development for lamivudine in combination with other drug . But very few method were reported the estimation of lamivudine in tablet dosage form for UV method exists for the estimation and determination of lamivudine in tablet dosage form. The aim of the study was to developed a simple precise, linear, economic and accurate UV method for determination of lamivudine in tablet dosage form.

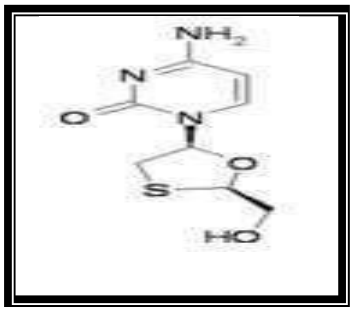

Fig. no. 01 structure of lamivudine

MATERIALS AND METHOD

➢ Instruments

Shimadzu double beam UV- spectrophotometer 1700 Ultra with matched pair, Qurtz cell corresponding to 1cm path length and spectral bandwidth of 1nm, Bath sonicatore and citizen weighing balance. Melting point apparatus of Equiptronics were used.

➢ Material

Lamivudine was a obtained sample. Lamivudine tablet were procured from a local pharmacy Dimethylformaide (DMF) was chosen as a solvent.

• Method development

➢ Standard stock solution

Standard stock solution were prepared by weigh 100mg of lamivudine transfer 100 ml volumetric flask about 10 min to solubilising the drug made up the volume was solvent to the mark with Diamethylformaide to get concentration of 1000 µg/ml.

➢ Sub stock solution

Took a 1 ml withdraw with the help of pipette out from standard stock solution was lamivudine transferred into 100ml volumetric flask separately and diluted up to 100 ml DMF that concentration of 1000 µg/ml.

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- **Determination of lamda max**

Standard solution of 100 µg/ml of lamivudine were prepared separately from respective sub stock scanned in the spectrum mode from 200nm to 400nm.The maximum absorbance of lamivudine was observed at 276nm. The were scanned in the wavelength range 200-400nm and the overlain spectrum was obtained.100mg weighed the amount of lamivudine was dissolved into 100ml of the volumetric flask with DMF. Pipette out 1 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with DMF.

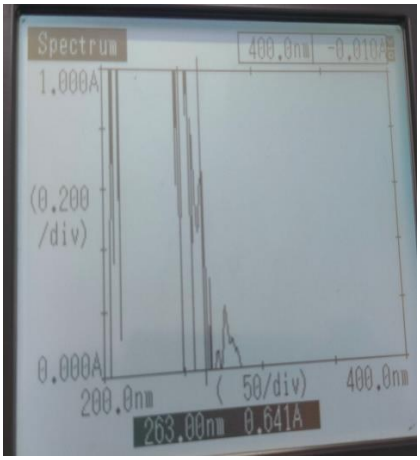

Fig. no. 02 calibration curve

Method of validation

The proposed method was developed by using linearity, accuracy, precision and recovery as per ICH guidelines.

- **Linearity and range**

Preparation of standard stock solution of Lamivudine 100 mg of lamivudine accurately weigh in to a clean and dry 100ml volumetric flask Dissolve with sufficient volume of DMF the volume made up to 100 ml distilled water obtained the concentration of 1000 µg/ml.

➢ **Working of standard solution**

From the working stock solution volume of 0.1, 0.2, 0.3,0.4, 0.5 ml were transfer in to 5 different 10ml volumetric flask the volume were made up to 10ml volumetric flask the volume were made up to 10 ml with DMF to obtained the concentration of 10%, 20%, 30%,40%, 50% µg / ml respectively.

- **ACCURACY**

The validity and reliability of proposed method were assessed by recovery studies by using standard addition method. The recovery of added standards (80%, 100%, 120%) was found at three replicate and three concentration level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method . Result of recovery study. To perform recovery study studies at 80% of the test concentration 10 mg of lamivudine was weighed and 8 mg of standard lamivudine was added the mixture was weighed 8mg of standard lamivudine was added the mixture was thoroughly . From this sample equivalent to 10mg of lamivudine was weighed and transferred to a 100ml volumetric flask . To it water was added to dissolve the sample. Finally the volume was made up to the mark with DMF . The solution was filtered through whatman filter paper. To the sample mixture was analysed. similarly recovery studies at 100%, and 120% of the

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test concentration were performed . The recovery study was performed three times at each level for the table formulation .

- **PRESICION**

Precision was determined by repeatability and intermediate precision (inter day) of drug . Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value SD and %RSD are less than 2 indicate the precision of method. Result of precision

Table no.1: Linearity studies

S. NO.	Concentration µg / ml	Absorbance
1.	10 µg / ml	0.669
2.	20µg / ml	1.156
3.	30µg / ml	1.660
4.	40µg / ml	2.093
5.	50µg / ml	2.669

Table no.02 : Recovery Studies

Tablet sample name	Table of recovery (%)	Amount present (mg/tab)	Amount taken (mg)	Amount of standard added (mg)	Total amount recovery (mg)	% recovery
Lamivudine	80%	100	10	8	16.43	100.01
		100	10	8	17.98	99.84
		100	10	8	18.43	99.92
	100%	100	10	10	18.09	99.49
		100	10	10	19.01	100.05
		100	10	10	19.76	99.88
	120%	100	10	12	20.08	100.49
		100	10	12	20.60	99.9
		100	10	12	21.06	100.58

Table no. 03 : Precision studies

Sample concentration	Sample sol. 1st	Sample sol. 2nd	Sample sol. 3rd	Mean	SD	%RSD
50µg / ml	0.601	0.604	0.607	0.60	0.0003	0.497
60µg / ml	0.607	0.616	0.609	0.61	0.0004	0.774
70µg / ml	0.701	0.701	0.700	0.70	0.0005	0.783

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Table no.04 :Solublity studies

S.NO.	Title	Result
1.	DMF	Freely soluble
2.	Analytical grade water	soluble
3.	Methanol	Slightly soluble
4.	Acetone	Practically insoluble

RESULT

- **Solubility of lamivudine**

Solubility test was passed as per criteria., Melting point of lamivudine was found to be 160-162^{0}C

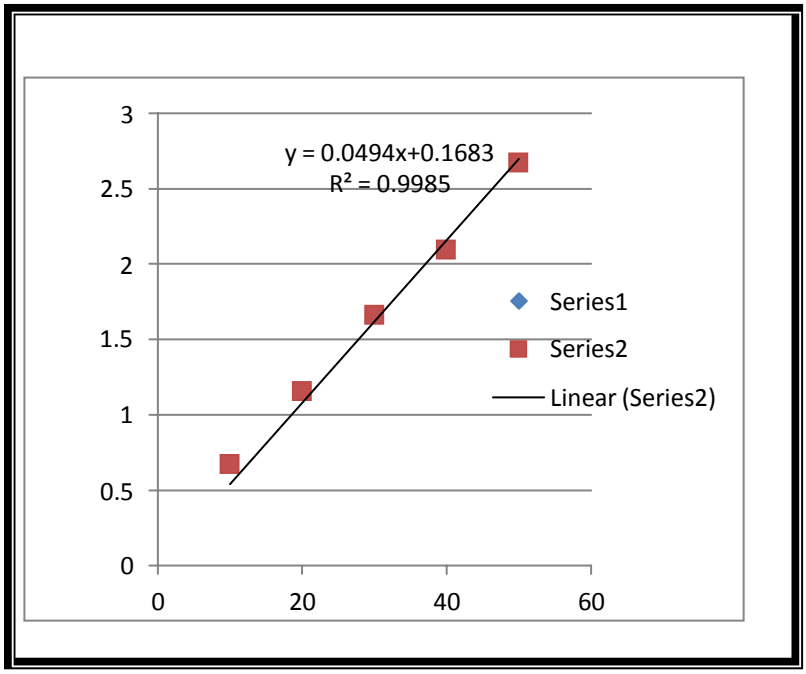

Fig. no. 03 Lamivudine standard curve

- **Result for linearity for assay method of lamivudine**

The linearity of method was determined at concentration level ranging from 10-50 µg/ml . The correlation coefficient value was found to be 0.9985.

- **Result for accuracy for assay method of lamivudine**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represent table .The high percentage recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

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- **Result for precision for assay method of lamivudine**
The % RSD for different sample of precision was found to be 0.686 and it is within acceptance criteria represented.

CONCLUSION

A new method is developed for method development of validation of lamivudine by UV spectrophotometer method .The sample preparation is sample . The analytical procedure is validated as per ICH Q2B guidelines and shown to be accurate, precise and specific. This method represents a fast analytical procedure for the simultaneous quantization of lamivudine. The method of amenable to the routine analysis of large number of sample with good precision and accuracy.

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