Method Development And Validation Of Lamivudine In Bulk And Tablet Dosage Form By Visible Spectroscopy Using NQS Reagent

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ABSTRACT

A Colourimetric Method for the analysis of LAMIVUDINE In Pure Form And In Tablets Has Been Developed Based On the Formation Of Reddish brown Coloured Complex When Sulphonate Group On NQS Reagent In The Presence Of NaOH Couples With Amine Group In Lamivudine. The Complex Exhibited Absorption Maxima at 453nm Obeying Beer's law In Range Of 10-60µg/ml. This Method Is Simple, Precise and Accurate With Recovery Of 99.8-102 %. The Line Equation Y = 0.0051x + 0.0647 With Correlation coefficient (r) Of 0.990 Was Obtained.

Keywords: Visible Spectroscopy, Lamivudine, NQS.

INTRODUCTION

Lamivudine is a prescription medicine approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV infection in adults and children 3 months of age and older. Lamivudine is always used in combination with other HIV medicines.

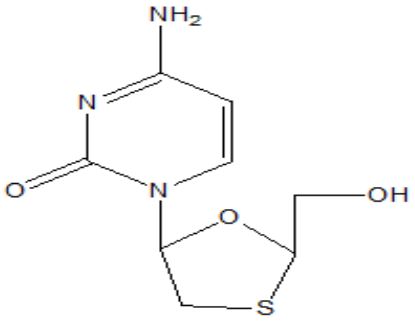
Lamivudine belongs to a group of HIV drugs called nucleoside reverse transcriptase inhibitors (NRTIs). NRTIs block an HIV enzyme called reverse transcriptase. By blocking reverse transcriptase, NRTIs prevent HIV from multiplying and can reduce the amount of HIV in the body.

HIV medicines can't cure HIV/AIDS, but taking a combination of HIV medicines (called an HIV treatment regimen) every day helps people with HIV live longer, healthier lives. HIV medicines also reduce the risk of HIV transmission.

Lamivudine (3TC), the negative enantiomer of 2'-deoxy-3'-thiacytidine, is a dideoxynucleoside analogue used in combination with other agents in the treatment of human immunodeficiency virus type 1 (HIV-1) infection and as monotherapy in the treatment of hepatitis B virus (HBV) infection. Lamivudine undergoes anabolic phosphorylation by intracellular kinases to form lamivudine 5'-triphosphate, the active anabolite which prevents HIV-1 and HBV replication by competitively inhibiting viral reverse transcriptase and terminating proviral DNA chain extension.

The pharmacokinetics of lamivudine are similar in patients with HIV-1 or HBV infection, and healthy volunteers. The drug is rapidly absorbed after oral administration, with maximum serum concentrations usually attained 0.5 to 1.5 hours after the dose. The absolute bioavailability is approximately 82 and 68% in adults and children, respectively. Lamivudine systemic exposure, as measured by the area under the serum drug concentration-time curve (AUC), is not altered when it is administered with food.

Lamivudine is widely distributed into total body fluid, the mean apparent volume of distribution (Vd) being approximately 1.3 L/kg following intravenous administration. In pregnant women, lamivudine concentrations in maternal serum, amniotic fluid, umbilical cord and neonatal serum are comparable, indicating that the drug diffuses freely across the placenta



Lamivudine

A Survey of literature reveals that few analytical methods were reported for Lamivudine by UV Method[4,8], HPLC[1,7,9], UPLC[5] in Bulk and it's Pharmaceutical Dosage form. However there is no method for the estimation of Lamivudine by colorimetric method using NQS as reagent.

MATERIALS AND METHOD'S

List of instruments :

- A SHIMADZU model PHAMASPEC-1800 UV-Visible double beam spectrometer with 1cm quartz cell was for recording spectra an absorbance measurements.
- WENSAR High precision balance
- Cuvettes – Quartz cells
- Digital ultrasonic cleaner.

Drug samples :

- Pure drug – Lamivudine received from Hetero lab, Hyderabad
- Formulation – Lamivudine (100mg) [Lamivir] were produced from market (Cipla pharmaceuticals)

Chemical reagents :

Reagents : All reagents used were of analytical grade were obtained from S.D fine chemical Mumbai.

- NQS (1,2-naphthaquinone-4-sulphonate sodium)
- NaOH (Sodium hydroxide)

Methods

Selection of wavelength

5mg of Lamivudine was accurately weighed and transferred to 50ml of volumetric flask. Solubilise the drug solution by distilled water and volume made up to 50ml with distilled water. Aliquot of the standard drug solution was pipetted into separate 10ml standard flask for preparing 10, 20, 30, 40, 50, 60 µg/ml of lamivudine. Mix well and add 2ml 1N NaOH and 1ml NQS reagent (0.7%). Allow to stand for 20 minutes, made up with distilled water. Absorbance was measured against 453nm for lamivudine. Overlay spectrum of lamivudine is shown in Fig:1

Preparation of standard stock solution

5mg of lamivudine was accurately weighed and transferred to 50ml volumetric flask, dissolved in distilled water and finally made up with 50ml distilled water to give standard solution of 100µg/ml of lamivudine.

METHOD VALIDATION

The method was validated using ICH guidelines by determining the following parameters :

Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of detection (LOD) and Limit

of quantification (LOQ).

Linearity

Five concentrations of the standard Lamivudine (10, 20, 30, 40, 50, 60µg/ml) were prepared and regression coefficients were found out.

Accuracy

The accuracy of the method was determined at the three percentage level 80%, 100%, 120%. The recovery and percentage relative standard deviation was found out.

Precision

To determine the precision of the proposed method, Pure drug solutions (lamivudine) at a concentration within the working range were prepared and analysed in three replicates during the same day on three consecutive days and results was found out.

Robustness

To evaluate the robustness of the methods, the concentration of sodium hydroxide and NQS reagent was changed and the effect of this change on the absorbance of the sample solution was studied. The results of this study was found out and it indicates that proposed method was robust.

Ruggedness

Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysis. From the %RSD values was found out, it concluded that the proposed method was rugged.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ values were calculated to check the sensitivity of the method by using following equations;

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

Where σ is the standard deviation and S is the slope of the curve.

RESULTS AND DISCUSSION

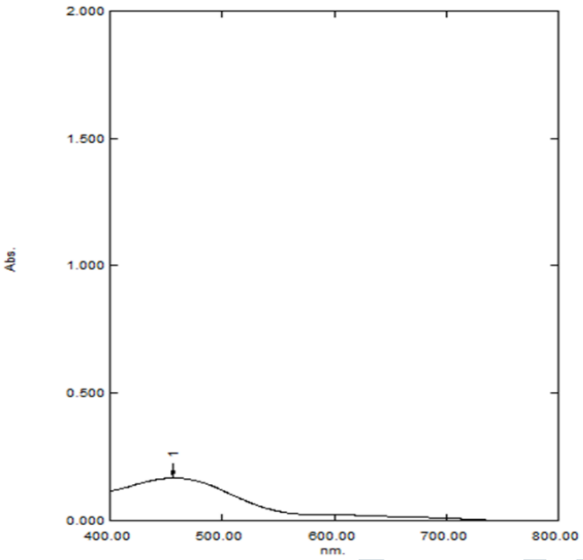


Figure:1 Visible spectrum of Lamivudine showing at λmax at 453nm

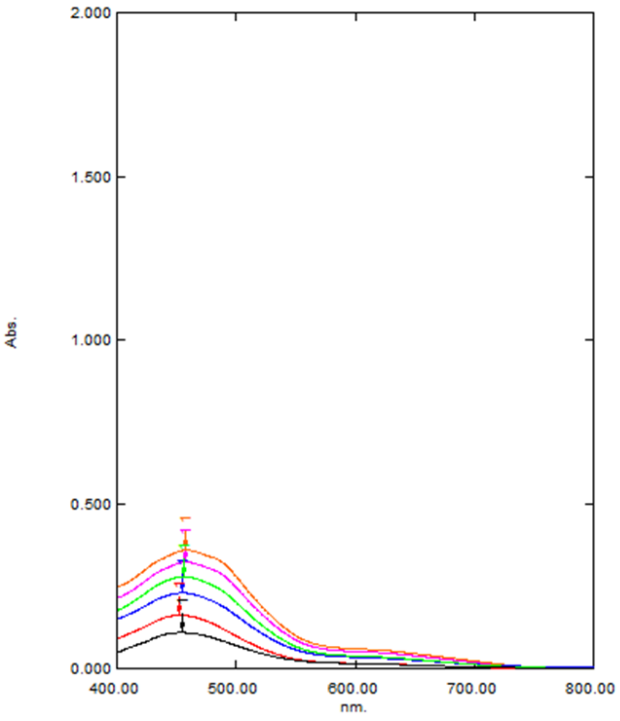


Figure:2 Overlay spectrum of Lamivudine

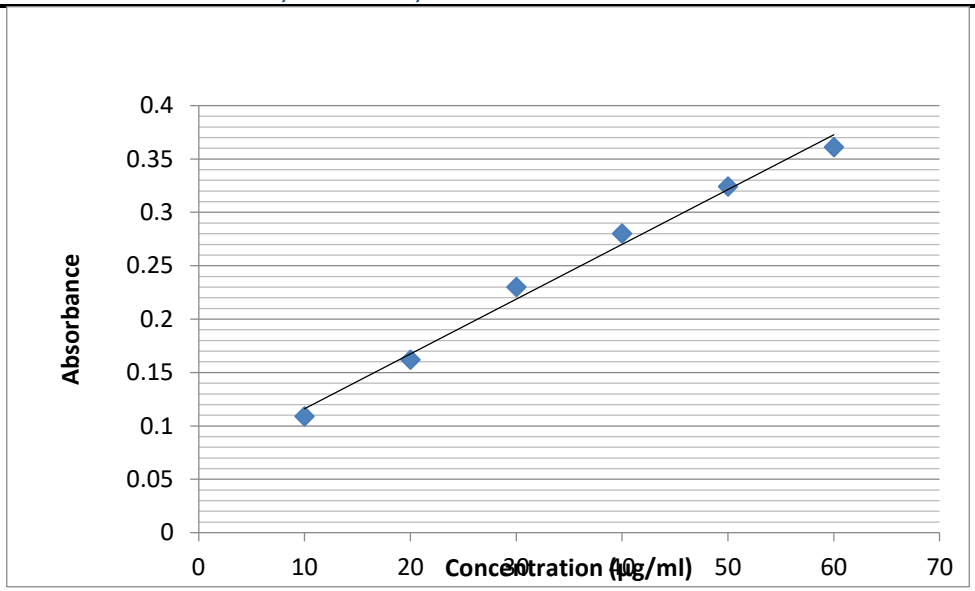


Figure :3 Calibration curve of Lamivudine at 453nm

Marketed Formulation	Drug	Label claim	Estimated Amount (mg)	% purity	% RSD
Lamivir	Lamivudine	100mg	100mg 99.8mg 100mg	100% 99.8% 100%	0.115

Table :1 Results of marketed formulation by colorimetry

DRUG	Theoretical % target level	Amount added (µg/ml)	Amount Recovered (mg)	% recovery mean	% RSD
LAMIVUDINE	80%	16	102.2	102.2	0.146
	100%	20	102.4	102.4	
	120%	24	102.9	102.9	

Table :2 Result of accuracy studies of lamivudine

DRUG	AMOUNT (µg/ml)	INTRA DAY		INTER DAY	
		% CONTENT	% RSD	% CONTENT	% RSD
LAMIVUDINE	20	98.4% 98.1% 98.3%	0.154	99.0% 99.3% 99.0%	0.174

Table :3 Result of Precision study of Lamivudine

DRUG	ANALYST	AMOUNT TAKEN (µg/ml)	AMOUNT FOUND (mg)	% CONTENT	% RSD
LAMIVUDINE	Analyst 1	20	100	100	0.173
	Analyst 2		99.7	99.7	
	Analyst 3		100	100	

Table :4 Result of Ruggedness study of Lamivudine

Sl. NO	AMOUNT TAKEN (µg/ml)	ABSORBANCE
1	10	0.109
2	20	0.162
3	30	0.230
4	40	0.280
5	50	0.324
6	60	0.361

Table :5 Result of Linearity study of Lamivudine

DRUG	AMOUNT TAKEN (µg/ml)	ALTERED PARAMETER		AMOUNT FOUND (µg/ml)	% CONTENT	% RSD
		1N NaoH	0.7% NQS			
LAMIVUDINE	20	1.5 ml	0.5 ml	100.0 100.1 100.2	100.0 100.1 100.2	0.05
		2.5 ml	1.5 ml	100.0 100.1 99.9	100.0 100.1 99.9	0.1

Table :6 Result of Robustness study of Lamivudine

L0D (Limit of detection) and LOQ (Limit of quantification)

DRUG	LOD(µg/ml)	LOQ(µg/ml)
LAMIVUDINE	6.93	21

Table :7 Result of LOD and LOQ of Lamivudine

ANALYTICAL DATA

PARAMETERS	LAMIVUDINE
Detection of wavelength	453 nm
Beer's law limit	10-60 (µg/ml)
Regression equation	Y=0.0051x+0.0647
Correlation coefficient	0.990
Slope	0.005
LOD	6.93µg/ml
LOQ	21µg/ml

SUMMARY AND CONCLUSION

The calibration curve for the determination of Lamivudine in solid dosage form was found to be precise, selective, rapid and it can be employed for the routine analysis. It could be precisely quantified and the entire calibration curve shows a linear relationship between the absorbance and concentration. Correlation coefficient was higher than 0.99. The low standard deviation and good percentage recovery indicate the reproducibility and accuracy of the method.

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