Ravi Kumar V. et al. / Vol 4 / Issue 1 / 2014 / 75-79.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

ESTIMATION OF LAMIVUDINE IN BULK AND FORMULATION BY TITRIMETRIC AND UV-VISIBLE SPECTROPHOTOMETRY

V Ravi Kumar, Yellanki Rupa*, M Chaitanya

Department of Pharmaceutical Analysis, Sri Indu Institute of Pharmacy, Ibrahimpatnam, Hyderabad, Andhra Pradesh, India.

ABSTRACT

Lamivudine is a synthetic chemotherapeutic agent used to treat HIV infection and hepatitis-B viral infection by inhibiting viral reverse transcriptase enzyme. So is very important to estimate the dosage form. There are so many analytical methods for the estimation of lamivudine. However there is need for developing an economical, fast and accurate method for estimation of lamivudine. The present study involved the analytical method of development of lamivudine by titrimetry and uv-visible spectrophotometry methods. Estimation of lamivudine in one marketed brand by titration and uv-visible spectrophotometric method.

Keywords: Lamivudine, Chloramines-T, Titrimetry, MBTH & PDAB reagent, UV-Vis. spectrophotometer.

INTRODUCTION

Chemically lamivudine is (2R,cis)-4-amino-l-(2-hydroxymethyl-l,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. Lamivudine has also been referred to as (-)2',3'-dideoxy, 3'-thiacytidine.Molecular formula of $C_8H_{11}N_3O_3S$ and molecular weight of 229.3 g/mol. Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/mL in water at 20°C and other polar solvents. It has the following structural formula:

5'-triphosphate, the active anabolite which prevents HIV-1 and HBV replication by competitively inhibiting viral reverse transcriptase and terminating proviral DNA chain extension. (2',3'-dideoxy-3'-thiacytidine,commonly called 3TC) is a potent nucleoside analogue reverse transcriptase inhibitor.

MATERIALS AND METHODS

All chemical, reagents & solvents are AR grade supplied by Quality traders, Hyderabad. The pure drug Lamivudine was obtained as gift sample from Cipla Laboratories, Hyderabad, India and sample tablets are purchased from local medical shop.

Instrumentation

Systronics 2202 UV/VIS double beam spectrophotometer, colorimeter EI-313 was used for the study.

Stock solution

Lamivudine stock solution was prepared accurately by weighing 1 mg of the drug and dissolving in 100 ml of de-ionized water thus making the concentration of 10µg/ml.

Test solution

For analysis of drug in tablet dosage form, twenty tablets were weighed accurately and triturated in the mortar to get fine powder. The amount of tablet powder equivalent to 1 mg of lamivudine was weighed and transferred to 100 ml volumetric flask and dissolved in deionized water. The solution was kept in ultrasonicator for 10 min and filtered through Whatman's filter paper No. 41.

MBTH Reagent: About 200mg of 3-methylbenzthiazolinone-2(3H)-hydrazone (MBTH) was accurately weighed and dissolved in 20ml of distilled water. The final volume was made up to 100ml

Corresponding Author:- Yellanki Rupa Email:- yellanki.rupa@gmail.com

with distilled water.

PDAB Reagent: 1gm of p-Dimehyl Amino Benzaldehyde was dissolved in 5ml H_2SO_4 and 5 ml of distilled water.

$FeCl_3$ solution

700m g of ferric chloride was dissolved in 100ml of 0.5%HCL.

Chloramine T (0.02M)

Chloramine-T solution (0.02M) was prepared by dissolving about 2.8 g of the chemical (Qualigens Fine Chem., Glaxo India Ltd., Mumbai) in water and diluting to 1 litre.

Sodium thiosulphate solution (0.02M)

Sodium thiosulphate solution (0.02 M) was prepared by dissolving about 5.0 g of the chemical (SISCO Chem, Industries, Mumbai) in 1 litre of water and standardized with pure potassium dichromate iodometrically.

Hydrochloric acid solution

Hydrochloric acid (5.0, 2.0 or 1.0 M) was prepared by diluting concentrated acid (s.d. Fine-Chem Ltd., Mumbai, Sp gr 1.18) appropriately with water.

Potassium iodide (10%) solution

Dissolve 100gms potassium iodide in 100 ml distilled water.

Starch indicator (1%) solution: Dissolve 1gm starch in 100ml of distilled water.

METHOD DEVELOPMENT

1) Titrimetric estimation

From stock solution different concentrations of 1-10mg of lamivudine was measured accurately into 100ml volumetric flask, add 5ml of 1M sulphuric acid then add 10ml of chloramines-T solution(0.02m),kept aside for 15mins with occasional stirring, then add 10% potassium iodide 5ml, starch as a indicator titrated against sodium thiosulphate, endpoint is indicated by colourless.

Drug content was calculated by the formula

Amount (mg) = (B-S)MW.R/2

B= vol of sodium thiosulphate consumed in blank.

S= vol of sodium thiosulphate consumed in sample titration

MW= mol. weight of drug

R= strength of chloramines-T (0.02m)

2) Colorimetric estimation

METHOD-1: PDAB reagent:

Aliquots of standard lamivudine solution (1m l=100µg) ranging from 0.5ml to 3ml were transferred to a series of 10ml volumetric flasks. To each volumetric flask added 2ml of PDAB reagent and 0.3ml of toluene and 0.02 ml of conc. H_2SO_4 and mixed well. The final volume was made upto 10ml with methanol. The rose red coloured complex was observed and absorbances were measured at 570nm against the reagent blank. The amount of lamivudine present in the sample solution was computed from its calibration curve.

METHOD-2: MBTH reagent:

Volumes of standard lamivudine solution (1ml=10µg) ranging from 0.5ml to 6ml were transferred in to series of 10 ml volumetric flasks. To each volumetric flask added 1.5 ml of MBTH reagent and 1.0 ml of $Fecl_3$ solution and mixed well. The final volume was made up to10ml with methanol and allows to stand for 10mins. The blue coloured complex was observed and absorbances were measured at 630 nm against reagent blank. The amount of lamivudine present in the sample solution was computed from its calibration curve.

3) UV spectroscopic estimation:

Standard preparation: Accurately weighed 100 mg of lamivudine test standard was transferred to a volumetric flask containing 25 mL of methanol solvent. This was sonicated for about 5 min to dissolve it and the resultant solution was diluted to 100 mL with methanol solvent. 10ml of this standard preparation was transferred to another volumetric flask and then diluted to 100 mL with methanol solvent.

Sample preparation: Ten tablets from the marketed sample were weighed and crushed uniformly with the help of a mortar and pestle. An accurately weighed powder sample equivalent to 100 mg of lamivudine was transferred into a volumetric flask containing 25 mL methanol solvent. The contents were sonicated for about 5 min so that the dissolution is enhanced and is completed in 15 min. 10ml of the supernatant solution was then taken and diluted to 100 mL with methanol solvent.

The baseline correction on the UV spectrophotometer was performed using methanol as blank solvent in both reference and sample quartz cells. The aim was to obtain the absorbance of the standard (10 µg/mL of lamivudine) and sample preparations at 270 nm and determine the content of $C_8H_{11}N_3O_3S$ in each tablet.

RESULTS AND DISCUSSION

Determination of lamivudine by titrimetry is done by using chloramines-T, Colorimetry using PDAB and MBTH reagents at 520nm and 505 nm & UV spectrophotometer at wavelength 270nm and beer law obeyed at 5-15microgram/ml.

Results for titrimetry-percentage purity = 95.01%

Colorimetry by PDAB reagent = 97.71%

MBTH reagent = 97.14%

UV spectroscopy method = 98.3%

Table 1. Titrimetric estimation values

Concentration(mg/ml)	Vol of sodium thiosulphate consumed in titration	LMV Amount found (mg)	Difference(mg)
Blank	19ml	0 mg	0mg
1mg/ml	18.8ml	0.458mg	0.542mg
1.5mg/ml	18.5ml	1.145mg	0.355mg
2mg/ml	18.2ml	1.832mg	0.168mg
2.5mg/ml	18.0ml	2.290mg	0.210mg
3mg/ml	17.8ml	2.748mg	0.252mg
4mg/ml	17.5ml	3.435mg	0.565mg
5mg/ml	16,8ml	4.638mg	0.362mg
6mg/ml	16.5ml	5.726mg	0.274mg
7mg/ml	16.0ml	6.870mg	0.130mg
8mg/ml	15.7ml	7.557mg	0.443mg
9mg/ml	15.2ml	8.702mg	0.298mg
10mg/ml	14.9ml	9.409mg	0.591mg

Average difference is 0.3491.
Percentage purity =7-0.3491/7*100=95.01%

Table 2. Colorimetric estimation values
METHOD-1: PDAB reagent:

Concentration(mcg/ml)	Absorbance at wavelength 520nm.
0.5ml	0.500
1.0ml	0.800
1.5ml	1.100
2.0ml	1.140
2.5ml	1.172
3.0ml	1.900
Sample	0.547

Percentage purity=0.557*0.004/1.140*0.002*100 = 97.71%

Table 3. METHOD-2: MBTH reagent:

Concentration(mcg/ml)	Absorbance at wavelength 505nm
1ml	19
2ml	23
3ml	27
4ml	35
5ml	39
sample	17

Percentage purity=17*0.2/35*0.1=0.9714*100=97.14%

Table 4. UV spectroscopic estimation values

Concentration(mcg/ml)	Absorbance at wavelength at 270nm.
5ml	0.454
8ml	0.900
10ml	1.107
12ml	1.304
15ml	1.507
Sample	0.590

Percentage purity=0.590*0.02*0.6/0.900*0.01*0.08=0.983*100=98.3%

Figure 1. Lamivudine

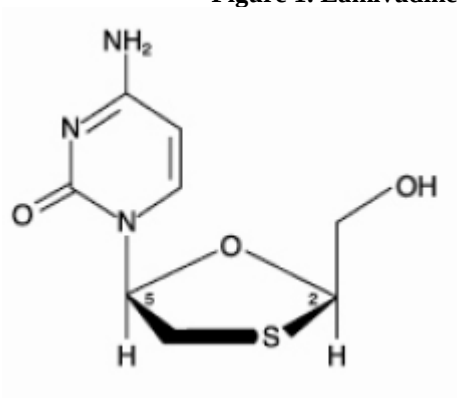


Figure 2. Colorimetric estimation

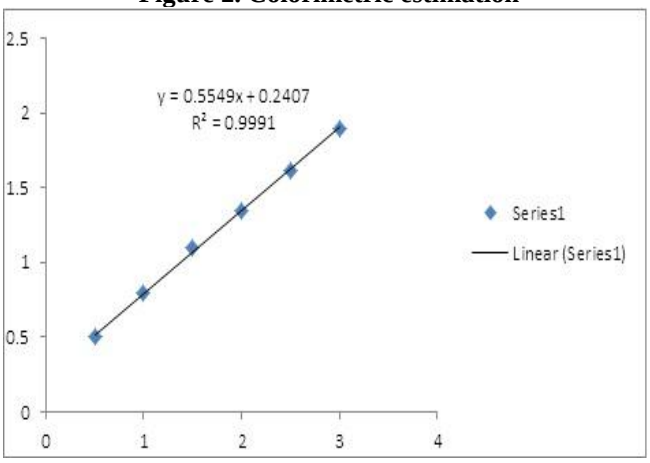


Figure 3. Calibration curve at 505nm

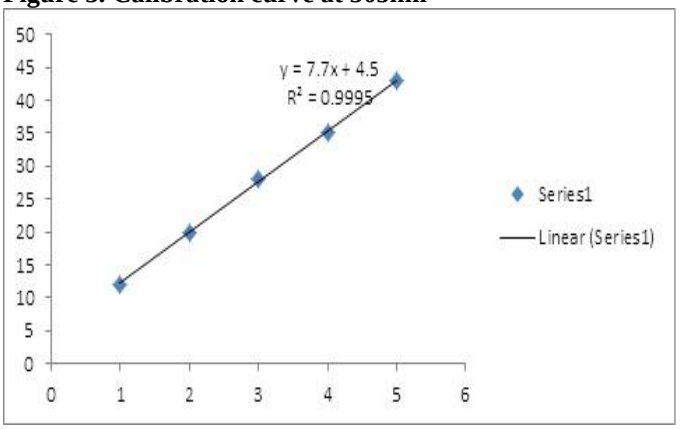
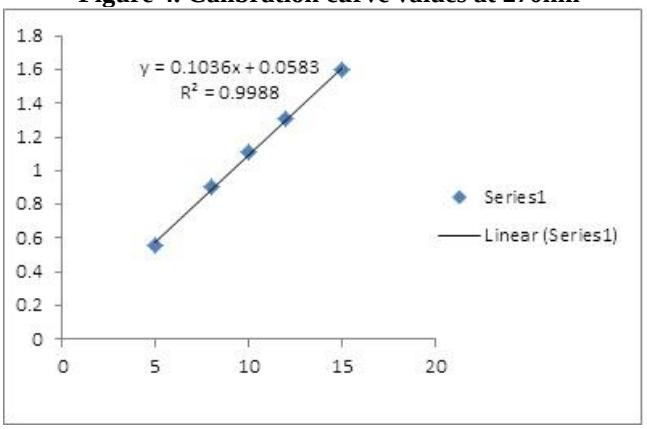


Figure 4. Calibration curve values at 270nm



CONCLUSION

For titrimetry 95% purity, colorimetry 97% purity and for uv spectroscopy 98% purity obtained. UV spectroscopy more precise in experimental since due to good accuracy, precision, sensitivity and it is therefore used for routine analysis.

ACKNOWLDGEMENT

We are thankful to our respective Chairman Mr.R.Venkat Rao, for providing us the instruments and also to our Principal Dr.A.Sambasivarao who given permission to carry out the work. We are also thankful to technical assistants who spared their time with us.

REFERENCES

1. Skoog DA, Holler FJ, Nieman TA. Principles of instrumental analysis. 5th ed. Philadelphia, Harcourt Brace College Publishers, 1998.
2. Beckett AH, Stenlake JB. Practical Pharmaceutical chemistry. 4th ed. London, Athlone Press, 1988.
3. Indian Pharmacopoeia 1996 (Addendum 2002) Govt. of India, Ministry of Health and Family Welfare, Controller of Publications. Delhi, 1996, 913.
4. Appalaraju S, Karadi Arvind B, Kamalapurkar OS. Spectrophotometric determination of Lamivudine. *Asian J Chem*, 14(1), 2022, 475-478.
5. Sarma CSN, Sastry CK, Sastry CSP. Determination of stavudine and lamivudine by visible spectrophotometry. *Acta Ciencia Indica Chem*, 28, 2002, 221-225.
6. V.p. Devmurari. Simultaneous spectrophotometric determination of lamivudine and abacavir in the mixture. *IJPSR*, 1(7), 2010, 82-86.
7. Zheng JJ, Wu ST, Emm TA. High performance liquid chromatographic assay for the determination of 2'-deoxy-3'-thiacytidine (lamivudine) in human plasma, *J Chromatogr B Biomed Sci Appl*. 761(2), 2001, 195-197.
8. Basavaiah, K Somashekar, BC. Titrimetric and spectrophotometric determination of lamivudine in pharmaceuticals. *IJCT*, 13(1), 2006, 7-12.

9. B. Uslu, S.A. Özkan. *Analytica Chimica Acta*, 466, 2002, 175–185
10. T.Sudha et al J. *Chem. Pharm. Res*, 2(5), 2010, 45-51.
11. Coates JAV, Cammack N, Jenkinson HJ, Jowerr AJ, Jowett MI, Pearson BA et al. 2'-deoxy-3'-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2 replication in vitro. *Antimicrob Agents Chemother*, 36, 1992, 773-778.
12. P HariPrasad et al. Simultaneous Estimation of Lamivudine and Stavudine by using RP-HPLC and Method Development as per ICH Guidelines, *IJPSR*, 3(7), 2012, 416-420.