Development and Validation of a Stability indicating HPLCmethod for the Determination of Lamivudine in Bulk and Pharmaceutical dosage form.

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ABSTRACT - A simple,selective, precise and accurate RP-HPLC method was developed and validated for the estimation of lamivudine in bulk and pharmaceutical dosage form. The determination was carried out on IntersilC-18 (250 X 4.6mm, 5µm) column using a mixture of Acetonitrile,water (50:50% v/v) as mobile phase with a flow rate of 1.0ml/min. The estimation was carried out at 270nm.The method was validated for linearity, accuracy, precision, specificity and robustness as per ICH norms. The retention time of the lamivudine was 2.6 min. The method shows linear responses in the concentration range of 40-120 µg/ml. with correlation coefficient (r2) of 0.999. This method can be useful for the estimation of lamivudine in its pure and tablet dosage forms.

Keywords: Lamivudine,RP-HPLC,Validation,Stability indicating, Stress condition.

Introduction

Lamivudin is L-2, 3-dideoxy- 3-Thiacyatidine having molecular [1] formula $C_8H_{11}N_3O_3S$. It inhibits both HIV Reverse transcriptase and also reverse transcriptase of hepatitis-B[2-4]. It is phosphorylated to active metabolites that compete for incorporation in to viral DNA. They inhibit the HIV-RTase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3-OH group in the incorporatednucleoside analogue prevents the formation of the 5, 3-phosphodiester linkage essential for DNA chainelongation therefore the viral DNA growth is terminated[5,6]. There are some methods reported for estimation of lamivudine by spectroscopy, [8,9,11] HPLC [10,12,13] and Lc-MS [14] in single and some in combination dosage forms.The present study has been under taken in order to develop a new simple,rapid, efficient and reproducible RP-HPLC method for analysis of lamivudine in accordance with ICH guidelines.

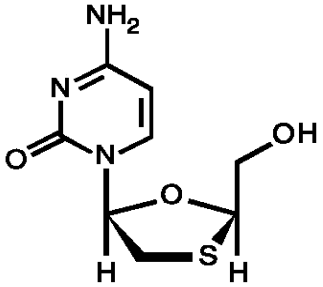


Fig1:structure of Lamivudine

MATERIALS AND METHODS

Chemicals and reagents:

Lamivudine obtained as a gift sample from Aurobindo Pharma Pvt Ltd, Hyderabad Acetonitrile, Methanol, Water, Potassium dihydrogen phosphate and Dipotassium phosphate are of HPLC grade. Pharmaceutical formulation of lamivudine was purchased from a local pharmacy.

Chromatographic conditions:

Chromatographic separation was performed at ambient temperature on a reverse phase intersil(C18) column, 250 mm × 4.6 mm i.d., 5 µm particle size. The mobile phase used in this analysis consists of a mixture of Acetonitrile and water in the ratio of 50:50. The mobile phase was filtered, degassed before use. The flow rate was adjusted to 1.0ml/min, the detector wavelength was set at 270nm. The injector volume of standard and sample was 20µl. The solution was injected and chromatograms were recorded. Calibration curve was constructed and regression equation was calculated for Lamivudine

Preparation of standard solution:.

Accurately weigh 50mg lamivudine of reference standard and transfer into 50ml volumetric flask, and add about 30 ml of diluent, sonicated for 10mins to dissolve properly. Then it is diluted to 50ml and mixed properly to get standard concentration of 1000µg/ml.

Preparation of sample solution:

Weigh and powder 20 tablets. Accurately weigh quantity of powder equivalent to 50mg of Lamivudine and transfer into 50ml of volumetric flask and add 30 ml of diluent , sonicated for 10mins to dissolve properly. Then it is diluted to 50ml and mixed properly to get sample concentration of 1000µg/ml. Aliquots of the solution was diluted to get a final concentration of 40, 60,80, 100and 120 mcg/ml of lamivdine .

Results and Discussions

Method validation

System suitability

System performance parameters of HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N) , tailing factor, Retention time(RT) were determined. .From system suitability studies it is observed that %RSD values are within the limit i.e not more than 2 which indicates good performance of the system. Chromatogram is shown in Figure: 2 and results are tabulated in Table No1.

Linearty

A series of solutions were prepared using lamivudine working standard solution at *a* concentration levels from 40-120µg/ml and the peak area response of all solutions are measured. A graph was plotted against the Concentration(µg/ml)on X-axis versus area/response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. linearity graph is shown in Figure: 3, linearity results are tabulated in Table No1(a).

Specificity

It is the ability to asses unequivocally the analyte in the presence of components that may be expected to be present. Excipients that are commonly used were spiked into a pre weighed quantity of drugs. Appropriate dilutions were injected into chromatographic system and the quantities of the drugs were determined. The results are tabulated in table No:5a &b.

Precision

Precision studies were performed. The results are reported in term of Relative standard deviation. The repeatability studies were carried out by estimating response of 6 different concentrations of lamivudine and reported in terms of % RSD. The results are tabulated in Table No:2.

Accuracy

Accuracy of the method was determined by calculating the recovery of lamivudine by the spiked method. Known quantity of Lamivudine was added to a pre-determined sample solution and the amount of Lamivudine was estimated by measuring peak areas. Mean % recovery values are within the limit(limit is 98-102%). Accuracy data was presented in Table No:3

Robustness:

Robustness is a measure of its capacity to remain unaffected by small , but deliberated variation in the method parameters and gives an indication of its reliability during normal The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for lamivudine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Lamivudine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The method passed robustness test with well % RSD. .Robustness data was presented in Table No:4

Results and Discussion

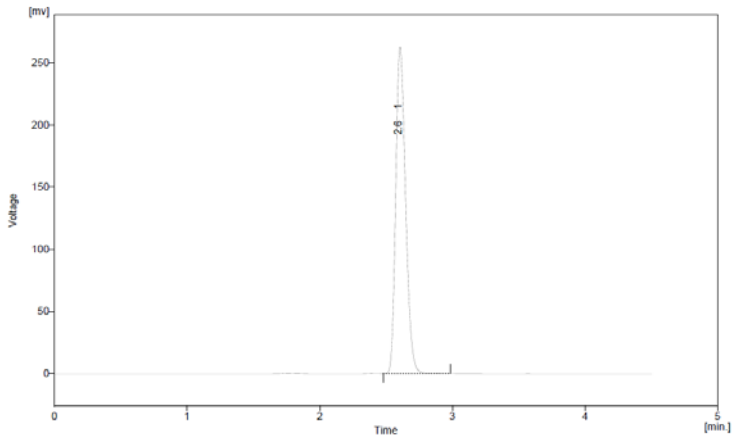
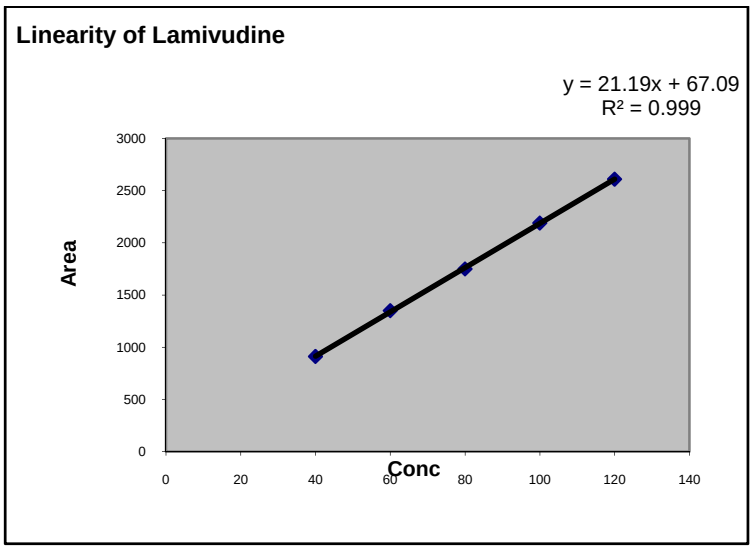


Fig2:Chromatogram of Lamivudine

Table No1:

S.No	Peak name	RT	Area(mV.s)	Height(mV)	USP plate count	USP Tailing	Area(%)
1	Lamivudine	2.603	1450.011	262.394	5407	1.50	100.000



Fig;3 Linearity Graph

Table No1 (a):Linearity

S..No	Conc .(µg/ml)	Peak area(mV.s)
1	40	911.212
2	60	1350.222
3	80	1750.042
4	100	2190.044
5	120	2610.412

Table No2: Precision

S.No	RT(min)	Area(mV.s)	Height(mV)	USP plate count
1	2.603	1790.220	346.575	5407
2	2.613	1790.189	347.347	5448
3	2.607	1788.089	344.587	5882
4	2.613	1790.410	348.929	5448
5	2.603	1790.118	348.090	5407
6	2.603	1787.215	346.084	5407
Mean	2.607	1789.8052		
Std dev	0.005	0.97		

Table No3:Accuracy data

Sample No	Spiked level	Peak Area	Amount spiked(mcg)	Avg. Amt Recovered(mcg)	% Recovery	Mean % Recovery
1	60	1450.011	6	64.88	98.14	98.31
2	60	1460.004	6		98.33	
3	60	1470.201	6		98.46	
1	80	1882.112	8	86.36	98.03	98.13
2	80	1893.11	8		98.14	
3	80	1892.114	8		98.23	
1	100	2400.113	10	108.21	98.25	98.37
2	100	2360.012	10		98.36	
3	100	2349.122	10		98.56	

Table No4:Robustness

Parameters used	Peak Area	RT	Theoritical plate	Tailing factor
Less Flow rate of 0.9mL/min	2020.006	2.887	5669	1.33
More Flow rate of 1.1mL/min	1650.220	2.373	5309	1.47
Wavelength at268nm	1800.004	2.60	5407	1.42
Wavelength at 272nm	1830.113	2.60	5407	1.37

Table No5a:Specificity: (Standard)

S.No	**Injection**	**RT**	**Area**	**Height**	**Tailing**	**USP Plate Count**
1	1	2.603	1800.422	350.440	1.50	5407
2	2	2.603	1810.009	351.732	1.42	5407
3	3	2.603	1820.112	352.539	1.50	5407
4	4	2.603	1810.205	351.806	1.42	5407
5	5	2.603	1810.216	352.361	1.42	5407

Table No5b: (Sample)

S.No	**Injection**	**RT**	**Area**	**Height**	**Tailing**	**USP Plate Count**
1	1	2.600	1760.223	322.853	1.53	4624
2	2	2.519	1780.414	326.078	1.40	4973
3	3	2.600	1780.201	328.235	1.40	4986
4	4	2.600	1770.401	328.424	1.47	4986
5	5	2.600	1760.323	323.562	1.40	4624

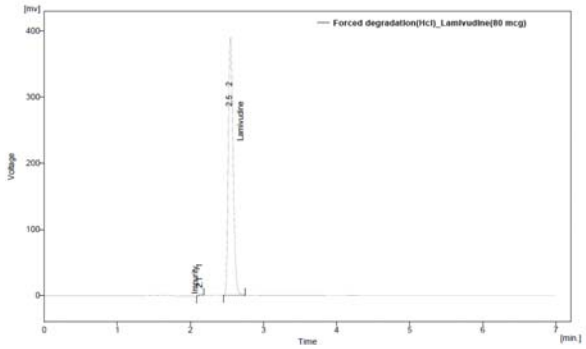


Fig3a: Forced degradation (Hcl)-Lamivudine

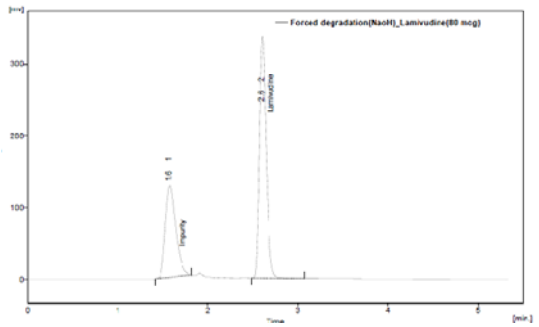


Fig3b : Forced degradation (NaOH)-lamivudine

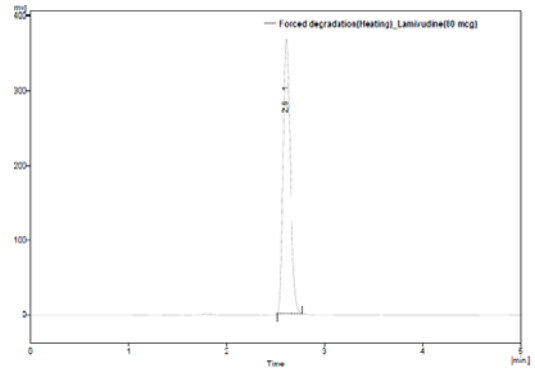


Fig3c**:** Forced degradation (Heating)-Lamivudine

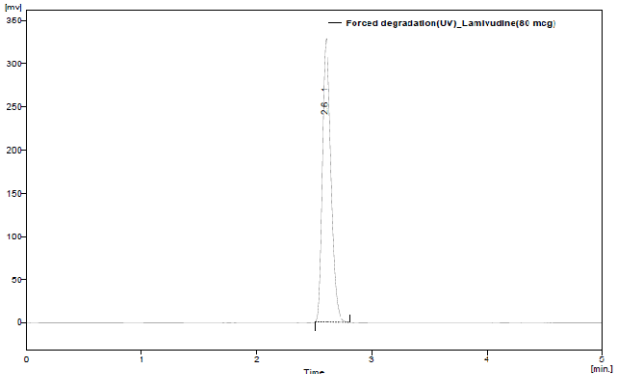


Fig3d : Forced degradation (U.V)) - Lamivudine

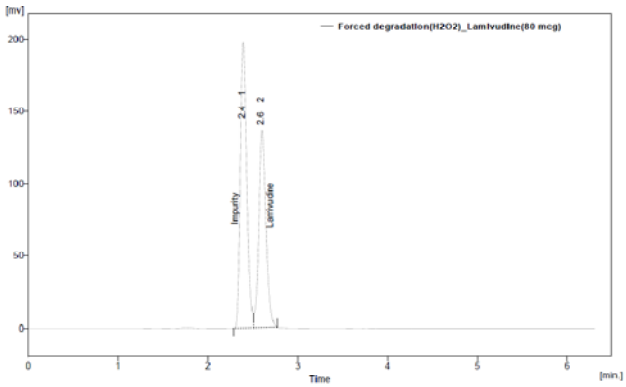


Fig3e : Forced degradation (H2O2))-Lamivudine

Table No6(a):Forced degradation (UV) -Lamivudine

S.No	RT (min)	Area (mV.s)	Height (mV)	Area (%)
1	2.603	1710.115	328.052	100.000
	Total	1710.115	328.052	100.000

Table No6(b):Forced degradation (NaOH) -Lalmivudine

SNo	RT (min)	Area (mV.s)	Height(mV)	Area(%)
1	1.577	1019.811	127.081	36.80
2	2.603	1751.772	337.491	63.20
	Total	2771.583	464.509	100.00

Table No6(c):Forced degradation (Heating) - lmivudine

S.No	RT (min)	Area(mV.s)	Height (mV)	Area (%)
1	2.607	1860.052	367.080	100.000
	Total	1800.052	367.080	100.000

Table No6(d):Forced degradation (Hcl) - Lamivudine

S.No	RT (min)	Area(mV.s)	Height (mV)	Area (%)
1	2.140	6.626	2.408	0.37
2	2.547	1761.132	389.541	99.63
	Total	1767.758	391.949	100.00

Table No6(e):Forced degradation (H2O2) - Lamivudine

S.No	RT (min)	Area(mV.s)	Height (mV)	Area (%)
1	2.393	1006.585	197.727	56.74
2	2.600	767.357	135.516	43.26
	Total	1773.942	333.516	100.00

Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lamivudine in bulk and pharmaceutical dosage form. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps Acetonitrile: Water (50:50% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The linearity range of Lamivudine were found to be 40-120 and linear regression coefficient was not more than 0.999. The values of %RSD were within 2 indicating accuracy and precision of the method. Validation of HPLC method as per ICH guidelines demonstrates that the method is simple, precise,linear, accurate, stability indicating and robust. This method can be used for the routine determination of lamivudine in bulk drug and in Pharmaceutical dosage forms.

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References:

[1] P.D.Senthi, HPLC: Quantitative Analysis Pharmaceutical Formulations, CBS Publishers distributors, NewDelhi (India), 2001,3-137.
[2] M.L. Bieganowska, A Petruczynik and A.Doraczynska-szopa. Thin leyered phase ion pair chromatography of Some Sulphonamides. Journal of Pharmaceutical and Bio-medical Analysis,1993,11,3,241-246.
[3] D .Paulson, T.Eastman, P.B. Bottini, Activity of Mefinide acetate 5% solution against Noso comial Pathogens[serial online}2004:3(7).
[4] R.C.Murphy,J.O.kucan, M.C.Robson, J.P. Heggers. The effect of 5% mefinide acetate solution on bacterial control in infected rat burns. The Journal of trauma.1980,23(10):878-81.
[5] K.Alekha Dashand Jennifer S. Harrison Ion- pair chromatographic method for the analysis of mefinide acetate. Journal of Chromatography, 1995, 78, 83-88.
[6] S.Howard,M.D.Yaffee,P.Donald,M.D.dressler, Topical application of mefinide Acetate arch dermatol 1969;100(3):277-281.
[7] D.Anantha kumar,G.Srinivasa Rao and J.V.L.N.Seshagirirao, "Determination simultaneous of lamivudine,Zidovudine and abacavir in tablet dosage forms by Rp-Hplc method".e-Journal of chemistry,2010,7(1),180-184.
[8] C.Josegnababu,and G.Vijayakumar "validate spectrophotometric estimation of lamivudine in pure and tablet dosage form" international Journal of Chemtech research,2009,1(4),1372-1375.
[9] Shalini.V,P.shanoojaa,S.Abdulammela,Basima.K,kharilala,HarishRajakb and V.ravichandrana Application of UV-Spectrophotometric methods for estimation of lamivudine in tablets, digest Journal of nanomaterial and biostructures,2009,4,(2)357-360.
[10] D.AnanthaKumar, M.V.NaveenBabu, J.V.L.N.SeshagiriRao and V.JayathirthaRaj, simultaneous determination of lamivudine and zidovudine and nevirapine in tablet dosage forms by RP-HPLC method" e-journal of chemistry 2010, 3 (1) 94-99.
[11] Gilcelia A., Cordeiro,a noemi Nagata,a jara Messerchmidt,a patricto Peraita-Zamora and N.C.Rodrigueb, "Multivariate Spectroscopic determination of the Lamivudine-Zidovudine" Journal of Brazil Chemical Society 2010 1-(7), 78-83.
[12] B. Jayakar, M. Kumar, C. Saravanan and M.V.Kumudhavalli, "Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Zidovudine" Journal of Chemicaland Pharmaceutical Research 2010, 2(1), 478-481.
[13] S.Jayaseelan, S.Suresh, G.Sathishkumar,V.Sekarand P.Perumal "Bioanalyticalmethod development and validation lamivudine by Rp-Hplc method International journal of chemtech research 2001 ,2(1) 163-167.
[14] Kathryn B. Kenney, Stephen A. WringRichard M. Carr Guy N. Wells and John A. Dunn, "Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem massspectrometry" Journal of Pharmaceutical and Biomedical Analysis 2000, 22, (6) 967-983.