International Journal of PharmTech Research

CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.13, No.02, pp 01-06, 2020

Analytical Method development and Validation of Lamivudine in Formulation by using Reversed Phase Ultra Performance Liquid Chromatography

Anshul kumar*, Chandana Majee, Vivek Namdev

Noida Institute of Engineering & Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh, India

Abstract : Aim of the experiment was to develop a simple, specific and accurate reverse phase ultra-performance liquid chromatographic (UPLC) method for the determination of lamivudine in the tablet dosage forms. The chromatographic separation was achieved on Acquity UPLC HSST3 (2.1 x 100mm) 1.8 um particle size and the mobile phase containing 0.1%TFA: MeOH for lamivudine. The run time was 10 min and the retention time of lamivudine was about 4.6. The detection was carried out 215nm using photo diode array detector (PDA) with a flow rate 0.6 ml/min. The linearity of lamivudine with correlation coefficient 0.9998. The recovery was found in the range (100±10%). The developed method was validated as per International Conference on Harmonization guidelines (ICH) with respect to specificity, linearity, accuracy, method precision, system precision, solution stability and robustness.
Key words : Lamivudine, method development, method validation, UPLC.

Introduction

Now a day ultra high performance liquid chromatography technique play a major role wordwide for the drug analysis purpose. [1,2]The main working principle behind the HPLC is based on the rate theory. The efficiency of the column cab be calculated by determine the column height (L) and numbers of theoretical plate(N). [4,5]Lamivudine (Lami),is an antiretroviral drug commonly called 3TC, used to prevent and treat HIV/AIDS also used to treat chronic hepatitis B and effective against both HIV-1 and HIV-2.[6] It is typically used in combination with other antiretrovirals such as zidovudine and abacavir. Lamivudine may be included as part of post-exposure prevention in those who have been potentially exposed to HIV.[7] Lamivudine is taken by mouth as a liquid or tablet. From, the various study it was found that lamivudine are some time used in the third trimester to reduce the risk of vertical transmission of hepatitis B virus (HBV).[8] The Joint United Nations Programme on HIV/AIDS (UNAIDS) has set a global target to increase uptake of PrEP to more than 3 million people by 2020.[9]

DOI= http://dx.doi.org/10.20902/IJPTR.2019.130201

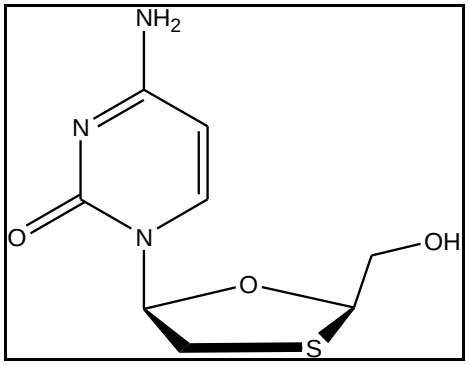

Fig 1. Structure of Lamivudine

Material and Methods

Lemovudin was found from the ……. All the other chemicals and reagents used were of analytical grade.

Preparation of mobile phase

Preparation of Buffer : Pipette out 1 mL of Tri FluoroAcetic Acid in 1000 ml of milli Q water and sonicate for 5 min.[10]

Preparation of Diluents : Methanol (100%)

Preparation of blank solution : Methanol.

Preparation of Standard Solution:Weighed accurately and transferred about 25.5 mg of lamivudine standard in a 50 ml volumetric mix and sonicate. Pipette out 5 ml of this solution and volume make up to 50 mLwith diluent.

Preparation of sample : Weighed and transferred drug substance 46.2 mg (equivalent to 25 mg of standard) in 50 mL volumetric mix and sonicate. Pipette out 5 mL of this solution and volume make up to 50 mL.[11]

Linearity

Linearity of the drug was determine taking the concentration of 50, 100, 150, 200 and 250 ppm of working concentration. Injections of all concentrations were carried out tree time replicate. Calibration curve was prepared by plotting the mean peak area versus concentration as the result a linear graph will found.[12]The Linearity co-efficient of mean response which was plotted against respective concentration, was calculated. The results are summarized in Table-1 and Fig. 2.

Accuracy

Recovery of the assay method for Lamivudine was established by three determinations of test sample using Tablets at 50%, 75% , 100% , 125% ,150% concentration. Each solution was injected thrice (n=3) into UPLC system and the average peak area was calculated to obtain percentage recoveries. All the individual recoveries were ound to be between 94.63% to 97.95 %. All individual recovery levels were found to be within 0.34% to 1.08% (% RSD). The results are summarized in Table-2.

System Precision

Six replicate injections of standard solution were given and mean of all of these values gives rise to the RSD value obtained. According to USP. RSD should not be more than 2%.

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing carbamazepine standard stock with those of the test sample The specificity study reveals the absence of interference of impurities with the drug, since no extra peak appeared at the same retention time

Linearity : By plotting the various peak area with respect to various concentration got a liner at curve with the R^2 value 0.999 given in Table 1 and Figure 2.

Table1.Result of linearity (sample at different conc.)

Concentrations (ppm)	Peak area			
	Injection-1	Injection-2	Injection-3	Average
50	6377.28	6466.28	6417.34	6420.30
100	13141.55	13073.38	13102.43	13105.78
150	19406.48	19515.05	19391.51	19437.68
200	25861.29	25667.93	25861.78	25797.0
250	32204.15	31819.13	31799.67	31940.98

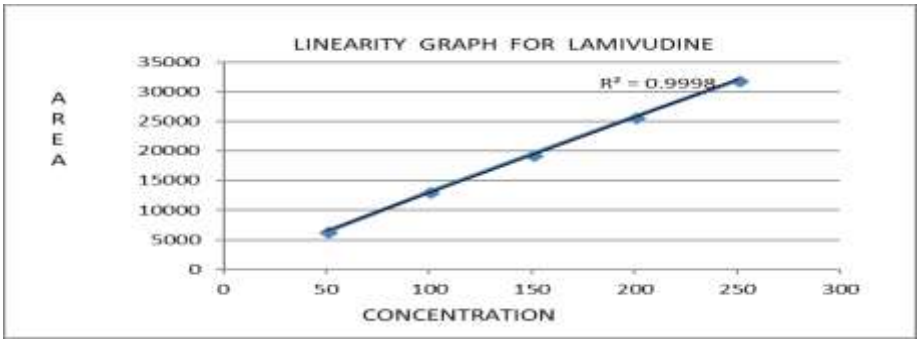

Fig .2 Calibration Curve of Lamivudine

Accuracy : All individual recovery levels were found to be within 0.34 to 1.24% (%RSD). The results are summarized in Table-2.

Table 2 :Calculation of Accuracy

Levels	Test Area	Average Area	Test Wt.(mg)	Standard Area	Standard Wt.(mg)	Amount Recovery (mg)	Amt Recovery %	SD	RSD
50%	3155.25	3230.66	12.20	6902.0	25.80	48.83	97.66	0.62	0.63
	3269.61								
	3267.12								
75%	4529.71	4519.26	17.80	6902.0	25.80	70.99	94.63	0.32	0.34
	4518.16								
	4509.90								
100%	6288.26	6259.78	23.96	6902.0	25.80	97.95	97.95	1.67	1.71
	6223.67								
	6267.41								
125%	7657.42	7687.48	30.13	6902.0	25.80	119.30	95.43	1.03	1.08
	7657.93								
	7747.10								
150%	8782.71	8678.32	34.43	6902.0	25.80	141.75	95.43	1.62	1.72
	8704.39								
	8547.86								

Result of system precision :

The precision of the system was evaluated by carryingout six independent injection of standard. The % RSD ofpeak area of the standard was found to be **0.13.** Theresults are summarized in **Table3**

Table 3: Result of system precision

Sl No	Replication	RT	Standard Are
1	Replicate-1	4.69	7124.86
2	Replicate-2	4.69	7134.17
3	Replicate-3	4.70	7123.03
4	Replicate-4	4.69	7112.32
5	Replicate-5	4.69	7108.06
6	Replicate-6	4.69	7118.81
Averag		4.69	7120.00
SD		0.0	9.34
%RSD		0.0	0.13

4: Result of method precision

S. No	Standard Area	Standard Weight(mg)	Test Area	Test Weight(mg)	Result (%)
Replicate 1.	7230.3	25.4	6396.9	46.1	99.1
Replicate 2.	7218.8	25.4	9447.9	46.5	99.0
Replicate 3.	7222.5	25.4	6543.0	45.5	102.6
Replicate 4.	7273.8	25.4	6484.4	46.1	100.2
Replicate 5.	7298.4	25.4	6378.1	45.0	101.2
Replicate 6.	7288.4	25.4	6604.6	46.1	102.3
Average	7249.0	25.4	6975.817	45.84	100.8
SD	-	-	-	-	1.56
RSD	-	-	-	-	1.55

Specificity

The specificity of the given sample given the table

Table 5 calculation of Standard of specificity

S. No	REPLICATES	R. T	AREA
1.	INJECTION-1	4.52	6883.85
2.	INJECTION-2	4.52	6853.34
3.	INJECTION-3	4.52	6884.81
4.	INJECTION-4	4.52	6879.28
5.	INJECTION-5	4.52	6887.80
6.	MEAN	4.52	6877.80

Robustness : Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.05 mL/min), wavelength content (±2nm).

- Wavelength are changed 217nm(Max) and 213nm(Min)
- Flow rate are changed 0.25ml (Min flow) and 0.35ml (Max flow).

The values of the robustness are given the table 6.

Table. 6 calculation of Robustness

S.No	Parameters Changes	Std Area	Std Wt (mg)	Test Area	Test Wt (mg)	Result (%)
Flow Rate	0.25ml	16940.3	25.0	15862.1	46.1	103.5
	0.35ml	12120.9	25.0	11255.3	46.1	102.7
Wave Length	213nm	14148.0	25.0	13215.7	46.1	103.3
	217nm	13396.7	25.0	12584.2	46.1	103.8

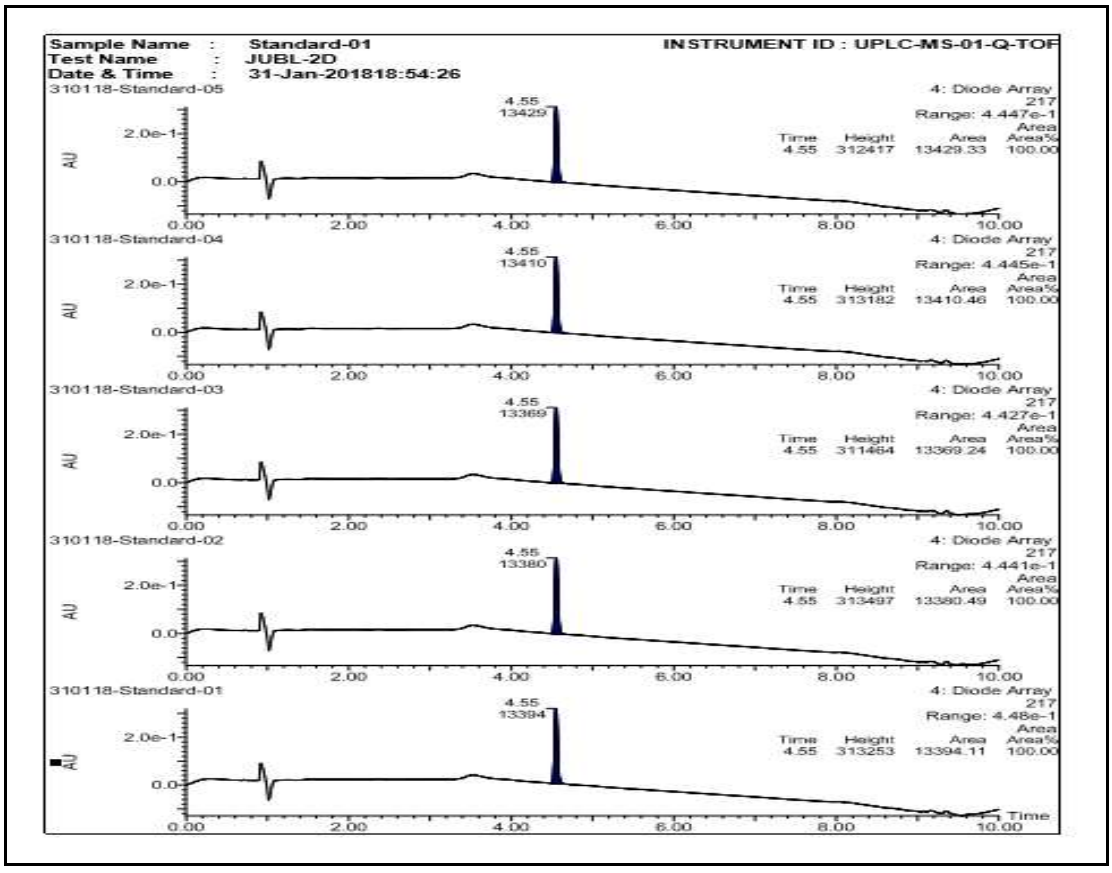

Fig.2. Chromatogram of standard at 217nm

Solution Stability

The Test solution and Standard solution of lamivudine was observed at time various intervals 0hrs, 8hrs, 16hrs, 24hrs was stable with good result.

Table.7 Calculation of stability solutions

Hrs.	Standard Area	Standard Wt.(mg)	Test Area	Test Wt.(mg)	Stability (%)
0	7550.95	25.10	6709.87	46.10	98.67
8	7170.15	25.10	6649.49	46.10	102.97
16	7290.76	25.10	6899.87	46.10	105.09
24	7240.85	25.10	6732.39	46.10	103.21

Conclusion :

In this study a New method has developed for the determine lamivudine proficiently and accurately with in a relatively short period by using Reverse phase UPLCMS method. It showed a good precision (RSD< 0.13%) and recovery (99.1% - 101.2%). and proved to be simple, linear, precise, accurate, robust, rugged and rapid. It gives faster elution, maintaining good separation more than that achieved with conventional HPLC. Short run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. The established analytical method can be used for routine analysis of lamivudine in bulk solid dosage forms

References

1. Beckett A.H. ; Stenlake J.B..,2004 .; Practical Pharmaceutical Chemistry. 4th ed. Churchill Livingstone, USA .; 275-298
2. Sharma B.K.(2004) Instrumental Methods of Chemical Analysis. 3rd ed. Krishna Prakashan, India. 113-115
3. Swartz M.E.2004 Ultra performance liquid chromatography: Tomorrow's HPLC technology today. LabPlus Int. ; 18(3): 6-9.
4. P. Saidulu, Sk.March – April 2016. Mastanamma Stability Indicating Gradient RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Abacavir and Dolutegravir in Bulk and their Combined Dosage FormInt. J. Pharm. Sci. Rev. Res., 37(2),; Article No. 43, Pages: 249-257 .
5. Bartholomew MM, Jansen RW, Jeffers LJ, et al.1997.; Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet 349:20-2.
6. Bartholomew MM, Jansen RW, Jeffers LJ, et al. Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet 1997;349:20-2.Armitage P, Berry G. Statistical methods.
7. Lai CL, Ching CK, Tung AKM, et al. Lamivudine is effective in suppressing hepatitis B virus DNA in Chinese hepatitis B surface antigen carriers: a placebo-controlled trial. Hepatology 1997;25:241-4.
8. Nevens F, Main J, Honkoop P, et al. Lamivudine therapy for chronic hepatitis B: a six-month randomised dose-ranging study. Gastroenterology 1997;113:1258-63.
9. Ling R, Mutimer D, Ahmed M, et al.1996.; Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology.24:711-3.
10. Madhusudhana reddy Induri1*, Bhagavan Raju Mantripragada2, Rajendra Prasad.March, 2016 YejellaDevelopment and validation of UPLC method for simultaneous estimation of Efavirenz and Lamivudine in pharmaceutical formulations Vol. 6 (03), pp. 029-033.
11. Wille, K., Noppe, H., Verheyden, K., Bussche, V., Wulf, E., 2014. Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater a*nalytical bioanalyical chemisry*, Volume 4, Issue 1, pp. 6-12
12. S. Walfish:2006 Analytical Methods: A Statistical perspective on the ICH Q2A and Q2B Guidelines for Validation of Analytics Methods, BIOPHARM International,: 1-6.

*****