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RP-HPLC method for determination of lamivudine in tablet

V.D.Patil[1], T.R.Chaudhari[2], U.S.Pendse[1], J.D.Fegade[2], R.Y.Chaudhari[2], N.O.Girase[1]*

[1]S.V.S. Science College, Dondaicha, Dist- Dhule, MS, (INDIA)

[2]College of Pharmacy, Faizpur, Dist-Jalgaon, MS, (INDIA)

E-mail : nogirase@gmail.com

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ABSTRACT

A simple, rapid, selective and precise RP-HPLC method was developed for estimation of lami vudine in tablet dosage form. Eurosphere-100 C_{18}, 250×4.6mm, 5µm particle size column, in isocratic mode with mobile phase ammonium acetate: methanol (90:10v/v pH- 3.8±0.1) was used. The flow rate was 1.0mL/min and component was measured at 277nm. The retention time of lamivudine was found to be 12.30min. Linearity for lamivudine was in range of 12.5-87.5µg/mL with correlation coefficient values 0.9997. The percentage recovery obtained was 99.58%. The proposed method is precise, selective and rapid for estimation of lamivudine.

KEYWORDS

RP-HPLC;
Lamivudine;
Method validation.

INTRODUCTION

Lamivudine is an acidic antiretroviral drug and chemically it is(2R-cis)-4-amino-1-[2-(hydroxyl-methyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone[1]. Various analytical methods have been reported in literature for estimation of lamivudine in single and in combination size exclusion and RP-HPLC[2], tandem mass spectroscopic [3], HPLC[4], HPLC/MS/MS[5-7], and raman spectroscopic[8].

Tablet dosage form of lamivudine was introduced recently and yet no one method is reported for the estimation of the same. The aim of present work is to develop a simple, rapid, precise and selective RP-HPLC method for the estimation of lamivudine in tablet.

MATERIAL AND METHOD

Instrument

High performance liquid chromatography system Chemitto LC 6600 equipped with universal injector with injection volume 20µL and Ultra-Visible (UV-Vis) detector. A Eurosphere-100 C_{18} column (250×4.6mm) 5µm particle size forms the stationary phase.

Chemicals and reagents

The gift sample of lamivudine was obtained from Atrix Pharma Lab, Hydrabad. Tablet Sachet of brand (Virolam, Ranbaxy) containing lamivudine 100mg was procured from a local pharmacy. All reagents used were of analytical grade: HPLC grade methanol and HPLC grade water was obtained from Qualigens.

Mobile phase

Ammonium acetate(0.025M) and methanol(90:10) mixture was used as mobile phase filtered through 0.45µ membrane filter and degassed for 20 minutes.

To HPLC grade water (900mL) ammonium acetate (1.93g) was added and shake for 30 minutes then make up volume up to 1000 mL with HPLC grade water to get ammonium acetate (0.025M) solution, pH was

adjusted to 3.8±0.1 with ortho-phosphoric acid.

Standard stock solution

Accurately weighed standard lamivudine(100mg) and transferred to a 100mL volumetric flask and dissolved with mobile phase. The flask was shaken for 30 min and volume was made up to 100mL with mobile phase to get lamivudine (10µg/mL) solution. Filtered through 0.45µ membrane filter and degassed for 30minutes.

Sample solution

Accurately weighed tablet powder equivalent to 100mg of lamivudine was transferred to 100mL volumetric flask containing 50mL mobile phase. Shake for 30min and volume was make up to the mark using mobile phase. The above solution was filtered through 0.45µ membrane filter and degassed for 30 minutes.

Assay

20µl of the test and standard solutions (n=3) were injected separately to an injector of HPLC and chromatograms were recorded. From the area, the amounts of both the drugs were calculated. The values are given in TABLE 1.

Linearity and calibration

From lamivudine standard stock solution, 0, 12.5, 25, 37.5, 50, 62.5, 75 and 87.5 µg/mL were prepared. Volume of 20µL of solution was injected into column with the help of hamilton syringe. Calibration curve of the area under curve vs. concentration was recorded.

Method validation

The analytical method was validated as per recommendations of USP[9] and ICH[10] guidelines for the parameters like recovery study, precision, ruggedness, robustness, specificity and system suitability test.

Recovery study

The accuracy of an analytical method is closeness of test results obtained by that method to the true value. The accuracy of an analytical method was established across its range. To known amount of standard solution of pure drugs lamivudine, standard drug was spiked at 50%, 100% and 150%. These solutions were subjected for analysis. Lower values of relative standard deviation (RSD) indicate the method is accurate. Values are given in TABLE 3.

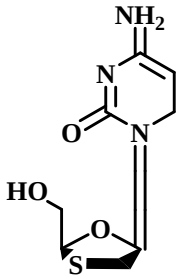


Figure 1: Chemical structure of lamivudine

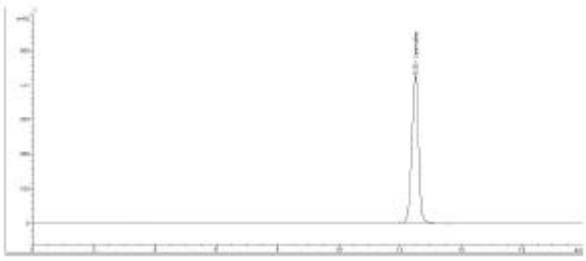


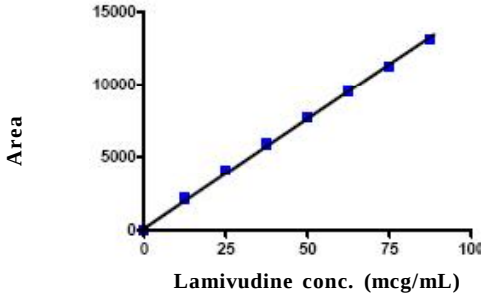
Figure 2 : typical chromatogram of the sample solution of lamivudine tablet at retention time of 12.30min

TABLE 1: Results of RP-HPLC assay

Tablet formulation	Actual concentration (mg)lamivudine	% Lamivudine ±RSD (n =3)
Virolam (Ranbaxy)	100	99.58± 0.1

TABLE 2 : Statistical data for linearity and calibration range

Sr. no.	Lamivudine conc. (µg/mL)	Area
1	0.0	0
2	12.5	2153
3	25.0	4051
4	37.5	5895
5	50.0	7681
6	62.5	9527
7	75.0	11249
8	87.5	13126



Precision

The precision of an analytical method is the degree

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TABLE 3 : Result for recovery studies

Sr. no	Level	Amount of standard drug added (mg)	Total amount of recovered drug (mg ±S.D)	%Recovery* ± SD	%RSD
1	50%	50	50.34±0.025	100.33±0.26	0.3
2	100%	100	100.07±0.034	101.8±0.17	0.2
3	150%	150	150.04±0.20	100.83±0.55	0.5
		Mean		101.1	0.8

*Mean of three observations

TABLE 4 : Results of precision studies

Lamivudine 10(µg/mL)	Intra-day precision Area under curve	Inter-day precision Area under curve
1	4125	4112
2	4120	4110
3	4122	4112
4	4120	4108
5	4118	4105
6	4115	4108
%RSD	0.031	0.049

of agreement among the individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample.

Variation of results within the same day(intra day), variation of results between days (inter day) were analyzed. Intra day precision was determined by analyzing, 10µg/mL of lamivudine for six times in the same day. Inter day precision was determined by analyzing, the same concentration of drug next day. The results are shown in TABLE 4.

Ruggedness

The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of condition, such as different laboratories, different analyst, different instruments, and different lots of reagent. The results for different analyst are shown in TABLE 5.

Robustness

Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

To determine robustness following variations are made in the analytical method

1. Flow rate; 2. Mobile phase composition; 3. Column temperature; 4. Mobile phase buffer

Specificity

Specificity is the ability to access unequivocally the analyte in the presence of components that may be expected to be present, such as impurities degradation product and matrix components.

A solution of 10µl of lamivudine was injected under the proposed chromatographic conditions to demonstrate the separations of lamivudine from its probable impurities. The drug was subjected to forced degradation by subjecting to it acidic (0.1 N and 1 N HCl), basic (0.1N and 1 N NaOH), Peroxide (3%), UV light, Sun light, Heat (120^0C), neutral hydrolysis.

System suitability test

As per USP-24 system suitability test was carried out on freshly prepared standard stock solutions of lamivudine. 20µl of the Lamivudine was injected under optimized chromatographic condition and following parameters was studied to evaluate the suitability of system.

- Number of theoretical plates (N)
- Retention Time (t_R)
- Tailing factor (T)
- Resolution (R)

The values of system suitability test were shown in TABLE 6

RESULT AND DISCCUSION

Lamivudine is synthetic aniretroviral agent. The market survey revealed that the above pharmaceutical dosage form is recently introduced in the market and literature survey also revealed that no methods are reported for the estimation of lamivudine in their tablet dosage form. Hence, an attempt has been made to develop the chromatographic method for estimation of lamivudine in Tablet.

A reverse Phase HPLC Method was developed for the estimation of lamivudine in tablet. The separation was achieved by a Eurosphere-100 C_{18} column and ammonium acetate: methanol (90:10v/v pH-3.8±0.1) as mobile phase, at the flow rate of 1.0 mL/min. the detection was carried out at 277nm. The retention time of lamivudine was found to be 12.30min. Linearity was assessed by a plot of concentration versus area, the graphs were found to be linear in the range of 12.5-87.5µg/mL for lamivudine with correlation co-

efficient values 0.9997.

On the basis of parameters fixed, the method of estimation was validated, for the following parameters:

Assay

The replicate analysis(n=3) of test and standard solutions by proposed method showed, the lamivudine content.

Recovery studies

Recovery studies were carried out by adding a known amount of standard solution of pure drugs (Lamivudine) to a preanalysed sample solution. These solutions were subjected for analysis. The study showed the result within acceptable limit.

Precision

Precision studies were carried out using parameter like Intra-day and inter-days precision, the study showed the results within acceptance limit, i.e. %RSD below 2.0, indicating reproducibility of the method(TABLE 4).

Ruggedness

Ruggedness studies were carried out only one parameters, i.e. analyst. Results showed that the %RSD was in the range of 0.1-1.4 i.e. less than 2, for different analyst. This study signifies the ruggedness of the method under varying conditions of its performance(TABLE 5).

TABLE 5 : Ruggedness studies

Tablet Formulation	Label claim (mg/mL)	Amount Found (%)	
		Analyst I	Analyst II
Virolam (Ranbaxy)	100	99.54	98.25

Robustness

TABLE-6 Change in flow rate

Change in flow rate	Resolution of lamivudine	Tailing factor	% RSD
Initial	2.55	1.029	0.08
-0.2	2.812	1.085	0.07
+0.2	2.470	1.486	0.09

TABLE 7 : Change in mobile phase composition

Change in mobile phase composition	Resolution of lamivudine	Tailing factor	% RSD
Initial	2.55	1.029	0.08
-2	2.647	1.070	0.06
+2	2.680	1.117	0.04

TABLE 8: Change in mobile column temperature

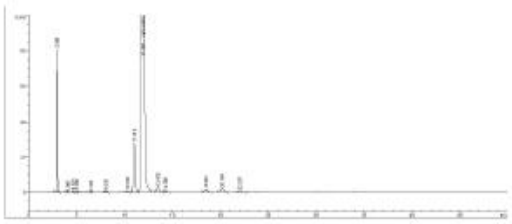
Change in column temperature	Resolution of lamivudine	Tailing factor	% RSD
Initial	2.55	1.029	0.08
-5	2.718	1.102	0.13
+5	2.331	0.892	0.05

TABLE 9 : Change in mobile phase buffer

Change in mobile phase buffer	Resolution of lamivudine	Tailing factor	% RSD
Initial	2.55	1.029	0.08
-0.2	3.054	1.047	0.10
+0.2	2.299	1.106	0.14

Specificity

Sr. no	Sample	Lamivudine area(%)	Degradation product area%
1	Initial	99.72	0.28
2	0.1 NaOH	97.99	2.01
3	1N NaOH	98.92	1.08
4	0.1 HCl	97.54	2.46
5	1 N HCl	86.53	13.46
6	3% hydrogen peroxide	90.52	9.48
7	UV light	99.64	0.36
8	Sunlighht	99.66	0.34
9	Heat ($120^{O}C$)	98.85	1.15
10	Neutral hydrolysis	97.65	2.35



System suitability test

TABLE 10 : System suitability test parameters

System suitability parameters	Proposed method
Retention time (t_R)	12.40
Theoretical plate number (N)	4055
Tailing factor (T)	1.038
Resolution factor (R)	2.8

CONCLUSION

The proposed RP-HPLC method is simple, accurate, rapid and selective. High percentage of recovery shows that the method is free from interferences of the excipients used in the formulations. Therefore method can be useful in routine quality control analysis of these drugs.

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REFERENCES

[1] S.Budavari, M.J.O'Neil, A.Smith, J.F.Kinneary (Ed.); In: 'The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals', 12th edn., Merck Research Laboratories, Merck and Co., INC. NJ, 5365 **(1996)**.

[2] K.C.Chimalakonda, H.K.Agarwal, A.Kumar, K. Parang, R.Mehvar; Bioconjung.Chem., **69**, 654 **(2007)**.

[3] A.K.Gehrig, G.Mikus, W.E.Haefeli, J.Burhenne; Rap.Commun.Mass.Spectrom., **21(16)**, 2704-2716 **(2007)**.

[4] M.I.Santoro, T.T.Fazio, A.K.Singh, E.R.Kedor-Hackmann; JAOAC, **90(3)**, 715-719 **(2007)**.

[5] H.N.Mistri, A.G.Jangid, A.Pudage, N.Gomes, M. Sanyal, P.Shrivastav; J.Chromatogr.B.Analyt. Technol.Biomed.Life Sci., **853(1-2)**, 320-332 **(2007)**.

[6] B.G.Pereira et al; J.Pharm.Biomed.Anal., **43(5)**, 1885-1889 **(2007)**.

[7] B.L.Robbins, P.A.Poston, E.F.Neal, C.Slaughter, J.H.Rodmann; J.Chromatogr.B.Analyt.Technol. Biomed Life Sci., **850(1-2)**, 310-317 **(2007)**.

[8] H.Rebiere, B.Mazel, C.Civade, P.A.Bonnet; J. Chromatogr.B.Analyt.Technol.Biomed.Life Sci., **850(1-2)**, 376-383 **(2007)**.

[9] The United States Pharmacopoeia-XXIV., Rock ville, 2149-2152 **(1998)**.

[10] International Conference on Harmonization, Draft guideline on validation of analytical procedures for pharmaceutical Availability, Federal Register, 59, 9750 **(1994)**.