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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND ZIDOVADINE IN TABLET DOSAGE FORM

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Abstract:

An accurate and precise HPLC method was developed for the Simultaneous determination of and Lamivudine. Separation of the drug was achieved on a reverse phase hypersil BDS C18, 100 X 4.6 mm, 5µ using a mobile phase consisting of Buffer and acetonitril in the ratio of 65:35v/v. The flow rate was 1mL/min and the detection wavelength was 270nm. The linearity was observed in the range of 25% to 150% with a correlation coefficient of not less than 0.998. The %RSD of and Lamivudine for In precision was found to be 0.15, 0.20 and 0.20, 0.05 respectively . The recovery was found to be 98% for and 102% for Lamivudine respectively which shows the accuracy of proposed method. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of and Lamivudine tablet dosage forms.

Key words: *, Lamivudine, Method development, RP- HPLC*

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1. INTRODUCTION:

Lamivudine 4- amino -1 -[(2R, 5S) -2 -(hydroxymethyl) -1, 3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one is a reverse transcriptase inhibitor used to treat HIV and hepatitis B infections. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV) to disrupt viral DNA synthesis. is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. Several methods have been developed using various chromatographic studies, and the scope of the present work is to expand and optimization of the chromatographic conditions, to develop RP-HPLC method. These drugs are evaluated for linearity, precision, accuracy, System suitability, Specificity, % Assay, Robustness, etc.

2. MATERIALS AND METHODS:

Lamivudine and Zidovadine pure drugs (API) received as gift sample from Hetero drugs Pvt Ltd. Combined form of Lamivudine and tablets was purchasd from the local market. Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, perchloric acid Ortho-phosphoric acid. All the above chemicals and solvents are purchased from Merck.

a. Preparation of Solutions:

i.Preparation of Standard solutions:

Accurately weighed 100mg of and Lamivudine and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of diluents. The volume made up to 100ml with diluents to obtain the concentration of 1000µg/ml for both the drugs. 0.1ml of stock solution was further diluted in a 10ml volumetric flask with mobile phase to get a concentration of 10 µg/ml.

ii.Samples Preparation

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 100 mg of active ingredient present in Lamivudine and was transferred into a 100 ml clean dry volumetric flask, Few ml of diluent was added to it and was shaken by magnetic stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 0.2 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as per with standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system.

iii.Cc standards;

Calibration curve standards were prepared by pipetting suitable aliquots from stock solution into separate 10 ml volumetric flasks and the volume was made up to the mark with diluent to obtain the CC standards in the range of 3.75 - 22.5 µg/ml and 50 - 300 µg/ml concentrations for Lamivudine and respectively.

b. Diluent: Mobile phase is used as diluent.

c. Chromatographic conditions:

A HPLC method had been developed for the simultaneous estimation of lamivudine & and validated using HYPERSIL BDS C18 (100 x 4.6mm, 5µm), mobile phase Acetonitrile: Buffer (35: 65v/v), pH of buffer adjusted to 2.5 with ortho phosphoric acid, detection wavelength at 270 nm at flow rate 1 ml/min.

2.4. System suitability:

These tests were based on the concept that the equipment, electronics, analytical operations and samples to be analysed which constitutes an integral system that can be evaluated as such. This test ensures that the analytical system was working properly and can give accurate and precise results The system suitability parameters were determined by preparing standard solutions of Lamivudine and . The solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

2.5. Method validation

The method validation was performed in accordance with ICH guidelines

2.5.1.Linearity

The linearity of the analytical method constitutes its ability to elicit test results which are directly proportional to the concentration of the analyte in the sample. Solution of different concentrations (level I - VI) were injected into the chromatographic system and measured the peak area. Chromatogram for Linearity of Lamivudine & from Level I — VI are given in Figure and Chromatogram Linearity report was given.

2.5.2. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often expressed as percent recovery by the assay of known added amounts of analyte. The standard solutions of concentrations 0f 50%, 100%, 150% of Lamivudine & were injected into chromatographic system. Calculated the amount found and amount added for Lamivudine & and also calculated the individual recovery and mean recovery values. The Chromatograms were recorded..

2.5.3. Precision

Precision of an analytical procedure is usually expressed the variance, standard deviation of coefficient of variation of a series of measurement. Precision of the method is determined in terms of System precision and Method precision The system precision was checked by using standard chemical substance to ensure that the analytical system was working properly. The retention time and area of six determinations was measured and percentage Relative Standard deviation was calculated. Method precision indicates whether a method is giving consistent results for a single batch, usually applied to standardization of methodology.

2.5.4. Robustness

The 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. The robustness of the proposed method was determined by analysis of aliquotes from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

2.5.5. Specificity:

The specificity was studied by establishing the interference of placebo with the drug. A sample of placebo was injected into the HPLC system as per the test procedure. Chromatogram of placebo should not show any peak at the retention time of analyte peak.

3. RESULTS AND DISCUSSION:

3.1 Assay of formulation:

A solution of 20µl standard, sample separately were injected into the chromatographic system and the peak areas of the Lamivudine & were measured and the percentage assay was calculated by using the formulae. Chromatograms were recorded measured the peak responses.

$$\text{Assay percentage} = \frac{A_t}{A_s} \times \frac{W_s}{D_s} \times \frac{D_T}{W_t} \times \frac{P}{\text{Avg. Wt}} \times \frac{}{100} \times 100$$

Label Claim

Representative chromatograms for standard, test and blank was given in figures 3,4 &5. Peak areas were given in table no. 1.

3.2 System suitability

System suitability parameters were determined according to ICH guidelines. Plate count was more than 2000, tailing factor was less than 2 and resolution was more than 2. All the system suitable parameters were passed and were within the limits. The results showing system suiability parameters were given in table no. 2

3.3 Validation

3.3.1.Linearity

The linearity was determined at six concentration in the range of 25 - 150 µg/ ml for both Lamivudine and for . The Peak areas against concentration were plotted and the calibration curve was constructed. The calibration curve was illustrated in Figure 3. The Correlation coefficient (r^2) was greater than 0.99 within the concentration range for both the drugs. The results for linearity were given in the table 3.

3.3.2. Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples (i.e.test Sample with known quantities of at the level of 50%, 100%, and 150% of target concentration. As the recovery results are found between 98.0% to 102.0%, the study proves that the method is accurate for the estimation The results for accuracy was given in the table 4.

3.3.3. Precision:

The precision of the method was studied by considering system precision and method precision. This method validation parameter was performed to ensure the closeness of results between true value and experimental value. The %RSD values of retention time and Peak area for five injections of Lamivudine & were found to be 0.15, 0.20 and 0.20, 0.05 respectively which are well within acceptance criteria limit. The method precision was performed to standardize methodology i.e., to check whether the developed method is precise. The values for Retention time and Peak area for six injections of lamivudine and were found to be within acceptance criteria. The results for precision were given in the table 5 & 6

3.3.4.Robustness:

This parameter was carried out to check the ability of the system to give unaffected results for small deliberate changes in system parameters and method parameters. Robustness of the method was studied by making deliberate changes in flow rate, mobile phase ratio. After making each change in the conditions, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit. Results were given in the table 7.

3.3.5. Specificity

The Chromatograms of Standard and Sample are identical with nearly same Retention time. No interference due to Placebo and Sample at the retention time of analyte which shows that the method was specific.

CONCLUSION:

A RP-HPLC method was developed and validated successfully for the estimation of lamivudine and in bulk and tablet dosage formulation. The analysis is

resolved by using a on HYPERSIL BDS C18 (100 x 4.6mm, 5µm), mobile phase Acetonitrile: Buffer (35: 65v/v), pH of buffer adjusted to 2.5 with ortho phosphoric acid, detection wavelength at 270 nm at flow rate 1 ml/min. The retention time for Lamivudine and were 2.15 min and 4.17 min respectively. The method was found to be accurate, precise, linear and reproducible for the simultaneous determination of lamivudine and in bulk and tablet dosage form (tablets). Hence these methods can be used for simultaneous estimation of Lamivudine and in routine table.

REFERENCES:

1. Anandakumar Karunakaran (2012). Analytical method development and validation for simultaneous estimation of Lamivudine and zidovudine. Eurasian J Anal Chemistry.volume; 56-66..
2. Anjaneyulu. N(2013). Validation for simultaneous estimation of Lamivudine. Asian Journal of Biomedical and Pharmaceutical Sciences. Vol 3 ,Issue 23, 7-11.
3. P. Vivek Sagar , T. Samidha, M. Vamshi Krishna and S. Shobha Rani. A Validated RP-HPLC method for simultaneous estimation of Aspirin and Prasugrel in tablet dosage form. International journal of Pharmaceutical Sciences and Research, 2014; 5(11): 1000-06.
4. Sindhura D (2013). Analytical Method Development and Validation for Simultaneous Estimation of Lamivudine, and Efavirenz by RP-HPLC.International Journal of Research in pharmacy and Biotechnology.pp;583-588.
5. Naga sandhya and Manikanta (2013).Deveiopment and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz .pp;147-155.
6. P. Vivek Sagar, S. Sushma, P. Shivani, and S. Shobha Rani. Stability indicating RP-HPLC Method for estimation of Agomelatine in tablet dosage form. International journal of Pharmacy and Biological Sciences, 2015; 5(4): 74-81

Table 1. Assay Data

LAMIVUDINE				
	STANDARD Rt.	SAMPLE Rt	STANDARD AREA	SAMPLE AREA
1	2.153	2.152	108.653	109.252
2	2.151	2.152	109.748	109.615
MEAN	2.152	2.152	109.549	109.280
%RSD	0.10	0.01	0.01	0.01
ZIDOVIDINE				
1	4.175	4.17	279.862	281.767
2	4.173	4.17	282.189	282.124
MEAN	4.174	4.17	281.903	282.375
%RSD	0.25	0.0	0.0	0.20

Table 2: System suitability parameters for Lamivudine and

SAMPLE	R_t	Peak Area	USP plate count	USP Tailing
LAMIVUDINE	2.152	109.615	1161	1.1
	4.174	282.124	1302	1.14

Table 3: Method Precision data of Lamivudine and

S.NO.	INJECTION NO.	LAMI		ZIDO	
		Rt.	AREA	Rt.	AREA
1	INJECTION -1	2.141	108.787	4.162	282.658
2	INJECTION -2	2.145	108.809	4.167	282.509
3	INJECTION -3	2.145	109.865	4.169	282.243
4	INJECTION -4	2.147	109.034	4.171	282.878
5	INJECTION -5	2.143	109.243	4.175	283.125
6	INJECTION -6	2.143	109.213	4.177	283.100
7	AVG	2.144	108.999	4.170	282.250

Table 4: System Precision data of Lamivudine and

S.NO.	INJECTION NO.	LAMI		ZIDO	
		Rt.	AREA	Rt.	AREA
1	INJECTION -1	2.141	108.787	4.162	282.658
2	INJECTION -2	2.145	108.809	4.167	282.509
3	INJECTION -3	2.145	109.865	4.169	282.243
4	INJECTION -4	2.147	109.034	4.171	282.878
5	INJECTION -5	2.143	109.243	4.175	283.125
6	INJECTION -6	2.143	109.213	4.177	283.100
7	AVG	2.144	108.999	4.170	282.250

Table 5: Robustness data of Lamivudine and

		LAMI	ZIDO
		Rt.	Rt.
Proposed Variation Of Flow rate	0.9ML/MIN	2.161	4.422
	1.1ML/MIN	2.137	3.880
Variation In Buffer Concentration Ratio	60 : 40	2.161	4.422
	70 : 30	2.137	3.880

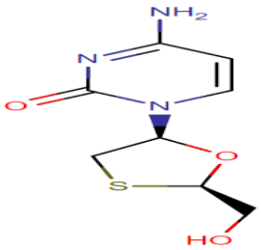

Fig 1: Structure of Lamivudine

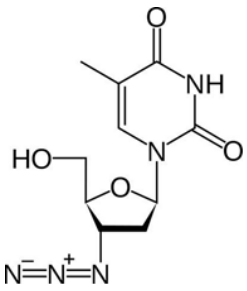

Fig.2: Structure of

Fig 3: Representative Chromatogram of working standard solution

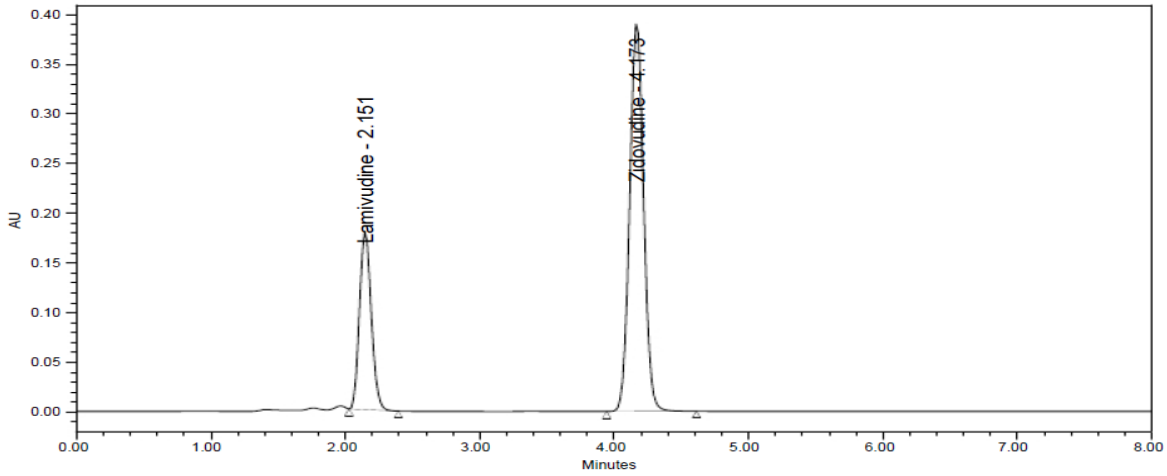

Fig 4: Representative Chromatogram of working sample solution