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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE IN BULK

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ABSTRACT

Objective: The scope of the present work is to expand and optimization of the chromatographic conditions and to develop RP-HPLC method.

Methods: The method was performed with various columns like C18 column, kromasil column, Hypersil BDS column. Among them, Symmetry C18 (250x4.6 mm, 5um) was found to be ideal as it gave good peak shape at 0.8 ml/min flow and validated for various parameters such as linearity, precision, accuracy, System suitability, Specificity, % Assay, Robustness, etc.

Results: The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Lamivudine and Zidovudine retention times and peak areas. The % RSD for the retention times of principal peak from 5 replicate injections of each standard solution were less than 2.0 %. The Linearity and correlation coefficient of Lamivudine and zidovudine was found to be 0.999. Precision was performed and % RSD for Lamivudine and Zidovudine were found to be less than 2.0. The % Recovery for each level was found to be the range of 98.0 to 102%. In Robustness, the % RSD of the peak area of all peaks for five replicate injections should be not more than 2.0.

Conclusion: Hence, the chromatographic method developed for Lamivudine and Zidovudine is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

Keywords: Lamivudine, Zidovudine, Optimization, Method Validation, RP-HPLC.

INTRODUCTION [1-4]

Lamivudine is a Nucleoside Reverse Transcriptase Inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). Zidovudine 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

High-performance liquid chromatography

High-Performance Liquid Chromatography [5-12] (HPLC) is the fastest growing analytical technique for the analysis of drugs. Chromatographic separation in HPLC is the result of specific interaction between sample molecules with both the stationary and liquid mobile phases. HPLC has been rapidly developed with the introduction of new pumping methods, more reliable columns and wide range of detectors. HPLC is also being automated which involve automated sampling, separation, detection, recording, calculation and printing of results.

HPLC offers a wide choice of chromatographic separation methodologies from normal to reverse phase and whole range of mobile phases using isocratic or gradient elution techniques. Various detectors available for HPLC are electrochemical detectors, refractive index detectors, fluorescence detectors, radiochemical detectors, mass-sensitive detectors and Ultra-violet (UV) detectors [13-14].

To develop a new HPLC method for any drug, knowledge of its molecular weight, polarity, ionic character, pK_a values, the wavelength of absorption, purity of compound and the solubility should be known. Method development involves considerable effort and time. The most commonly applied method is reversed phase and reverse coupled with ion-pairing. These two techniques probably account for more than 85% of the applications for a typical pharmaceutical compound. The typical pharmaceutical compounds are considered to be an active pharmaceutical ingredient of molecular weight of less than 1,000 Daltons. Depending on the number of active compounds to be resolved or separated, the more complex is the separation, the more gradient elution will be advantageous over isocratic mode. Optimization can be started only after reasonable chromatogram has been obtained.

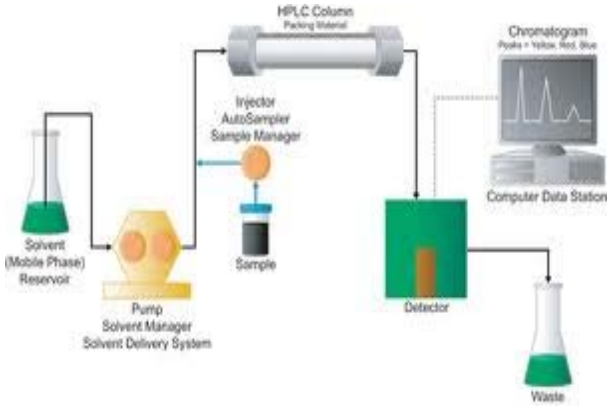


Fig. 1: Schematic diagram of HPLC instrument

The main objective was to develop a sensitive, simple, rapid, reliable and accurate analytical method for the simultaneous estimation of Lamivudine and Zidovudine in Bulk and validation of developed method by using RP-HPLC technique.

MATERIALS AND METHODS

Materials

Zidovudine, Lamivudine, Water (Milli-Q), Methanol from Merck.

Instruments

Table 1: Shows list of instruments

S. No.	Instrument	Model
1	HPLC	Shimadzu LC Software UV-VIS detector.
2	Column	Symmetry C18,250x4.5 mm,5um
3	Electronic balance	Mettler tolledo, XS-205DU
4	Pipettes and Burettes	Borosil
5	Beakers	Borosil

HPLC method development [15-18]

The objective of this experiment was to optimize the assay method for estimation of lamivudine and zidovudine-based on the literature survey made. Several trials were carried out and the optimized method was developed.

Preparation of standard solution

Accurately weigh and transfer about each 25.0 mg of Lamivudine and Zidovudine sample into a 25 ml volumetric flask, dissolve in and dilute to volume with diluent. Take 1.0 ml of this solution into 10 ml volumetric flask and makeup to the mark with diluent.

Optimization of column

The method was performed with various columns like C18 column, kromasil column, Hypersil bds column, Symmetry C18 (250x4.6 mm, 5um) was found to be ideal as it gave good peak shape at 0.8 ml/min flow.

Optimized chromatographic conditions

Chromatographic parameters

Equipment: High-performance liquid chromatography equipped with Auto Sampler and UV detector

Column: Symmetry C18 (250 x 4.6 mm, 5µm)

Flow rate: 0.8 ml/min

Wavelength: 250 nm

Injection volume: 20 µl

Column oven: 30ºC

Run time: 10 min

Chromatogram for standard was shown in fig. no.2 and for the sample in fig. no.3

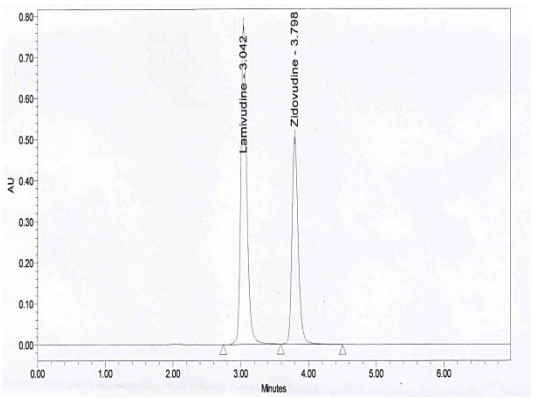


Fig. 2: Chromatogram for standard

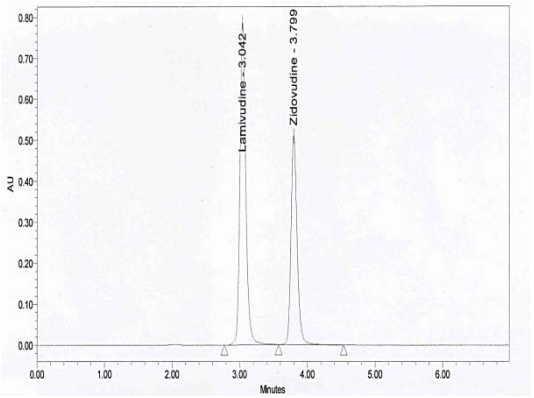


Fig. 3: Chromatogram for sample

Preparation of mobile phase

Prepare a degassed mixture of water and methanol in the ratio of 40:60 v/v

Diluent preparation

Use the mobile phase as diluent

Preparation of standard solution

Accurately weigh and transfer about each 25.0 mg of lamivudine and zidovudine sample into a 25 ml volumetric flask, dissolve in and dilute to volume with diluent. Take 1.0 ml of this solution into 10 ml volumetric flask and makeup to the mark with diluent.

Preparation of sample solution

Accurately weigh and transfer about each 25.0 mg of Lamivudine and Zidovudine sample into a 25 ml volumetric flask, dissolve in and dilute to volume with diluent. Take 1.0 ml of this solution in to 10 ml volumetric flask and makeup to the mark with diluent

Quantitative estimation

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times 100$$

Where:

AT = Average area of each main peak obtained from the chromatogram of the sample solution

As= Average area of each main peak obtained from the chromatograms of the standard solution

WS = Weight of each Lamivudine and Zidovudine in standard solution (mg/ml)

WT= Weight of each Lamivudine and Zidovudine in sample solution (mg/ml)

DS =Dilution of standard solution

DT =Dilution of test solution

P= potency of each standard

RESULTS AND DISCUSSION

Validation parameters

System suitability

Standard solutions were prepared by using Lamivudine and Zidovudine working standards as per test method and were injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Lamivudine and Zidovudine retention times and peak areas. Chromatogram for injection 4 and 5 were shown in fig. no. 4 and 5.

Acceptance criteria

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %

2. The number of theoretical plates (N) for Zidovudine and Lamivudine peaks is NLT 2000.

3. The Tailing factor (T) for Zidovudine and Lamivudine peaks is NMT 2.0.

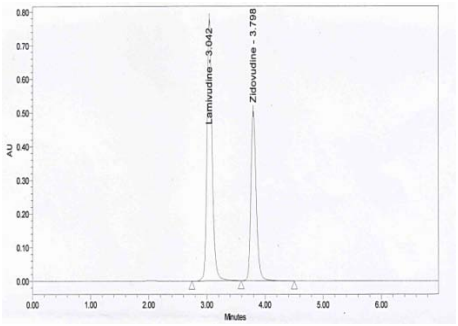


Fig. 4

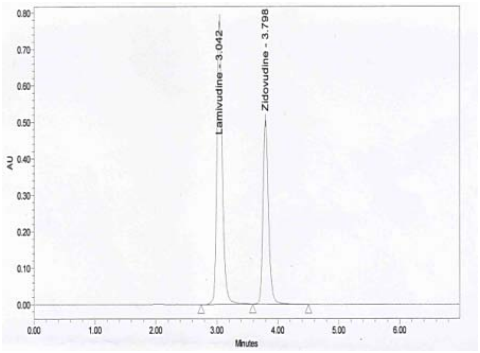


Fig. 5

Specificity

Lamivudine and Zidovudine solutions were prepared individually at a concentration of 0.1 mg/ml and spiked was also prepared. All these solutions were analyzed as per the HPLC method

Table 1 summarizes the retention time (RT) and the relative retention time (RT ratio) values obtained for Zidovudine and Lamivudine Chromatograms were shown in fig. no 6 and 7.

Acceptance criteria

- The peaks of blank should not interfere with peaks of Lamivudine and Zidovudine

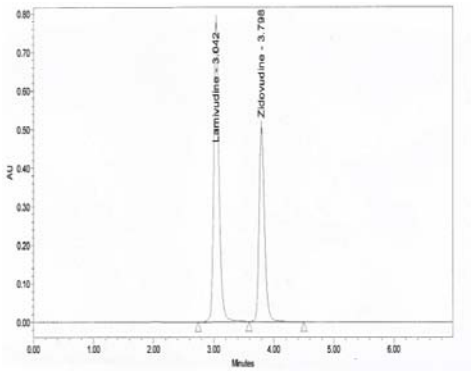


Fig. 6

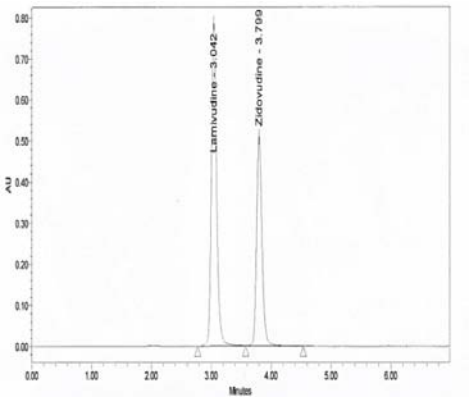


Fig. 7

Linearity

The linearity of the HPLC method was demonstrated by analyzing the solutions ranging from 0.080 mg/ml to0.120 mg/ml, which is equivalent to 80% to 120% of the Lamivudine and Zidovudine working strength.

Preparation of stock solution

Accurately weigh and transfer about each 25.0 mg of Lamivudine and Zidovudine standards into a 25 ml volumetric flask, dissolve in and dilute to volume with diluent.6

Preparation of Level–I: 0.8 ml of stock solution is taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level–II: 0.9 ml of stock solution is taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level–III: 1 ml of stock solution is taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level–IV: 1.1 ml of stock solution is taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level–V: 1.2 ml of stock solution is taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area. The linearity Results were shown in the fig. No.8 and 9 shows the line of best fit for concentration versus peak area of Lamivudine and Zidovudine.

Acceptance criteria

- Correlation Coefficient should be not less than 0.999.

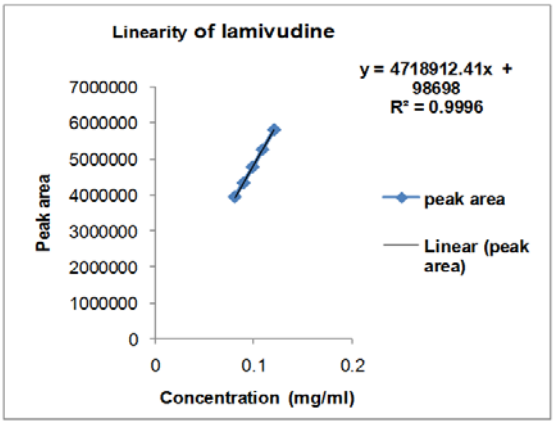


Fig. 8

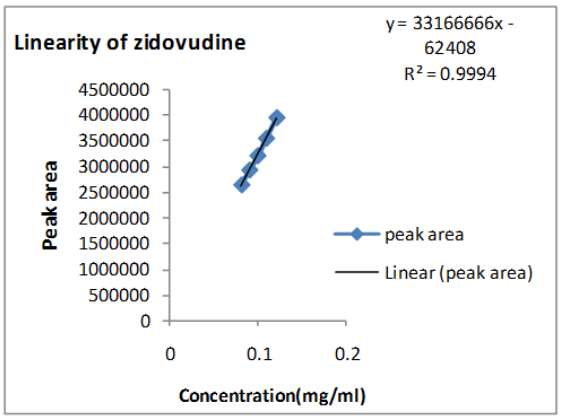


Fig. 9

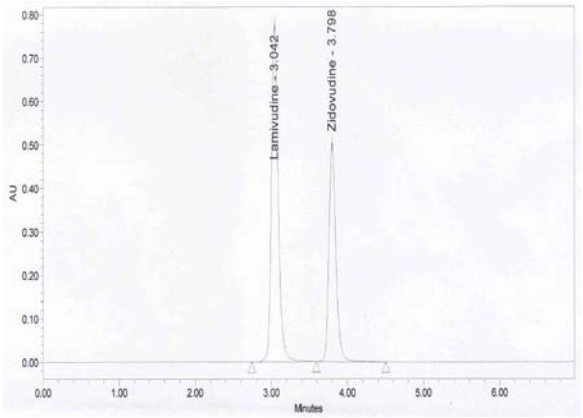


Fig. 10: Chromatogram for standard injection-5

Precision

Method precision

The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was calculated. One of the Chromatogram was shown in fig. no. 10.

Acceptance criteria

The relative percentage deviation for the assay values should be not more than 2.0.

Intermediate precision/ruggedness

Evaluating the variability of the assay results obtained with the analysis of Lamivudine and Zidovudine sample six times (six individual sample preparations) by different days, different reagents and different columns assessed the method ruggedness. One of the chromatograms was shown in fig. 11.

Acceptance criteria

a) The bias in assay values should be less than+or-1.5.

b) The percentage relative standard deviation of the assay results should be not more than 2%.

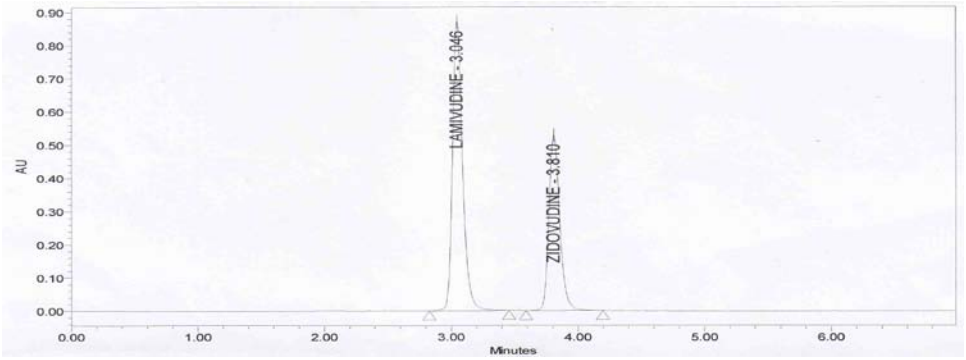
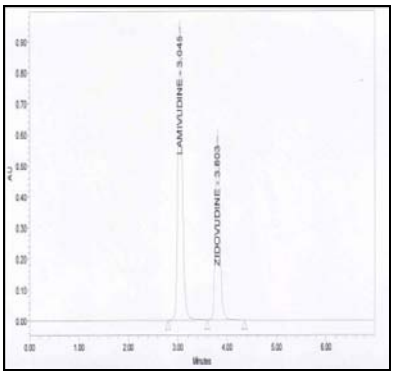


Fig. 11: Chromatogram for sample injection-6

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms were recorded for the same.



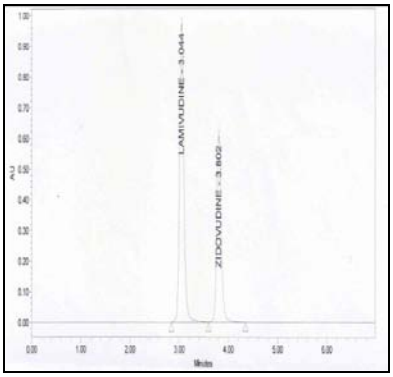


Fig. 12-13: Chromatogram for sample concentration-120%

Procedure

Inject the standard solution, Accuracy-50%, Accuracy-100% and Accuracy-150% solutions.

Calculate the Amount found and calculate the individual recovery and mean recovery values.

% Recovery= Amount found/Amount added × 100

Chromatograms were shown in fig. No. 12 and 13

Acceptance criteria

The % Recovery for each level should be between 98.0 to 102.0%

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied from 0.7 ml/min to 0.9 ml/min.

Results for actual flow (0.8 ml/min) have been considered from Assay standard.

b) The organic composition of mobile phase was varied about 10%

Acceptance criteria

The % RSD of the peak area of all peaks for five replicate injections should be not more than 2.0. Results of variation of flow were shown in table 2 & 3 and chromatograph shown in fig. 14-15.

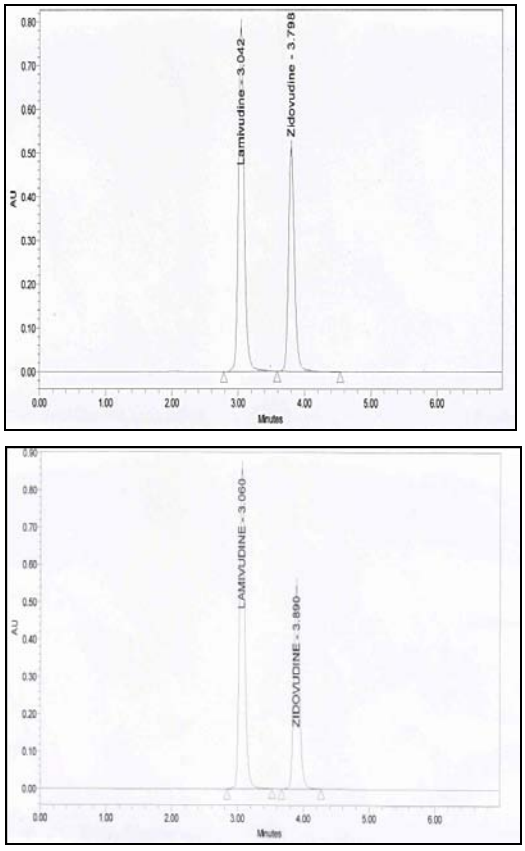


Fig. 14-15: Chromatogram showing actual composition and mobile phase-high

Table 2-3: Shows results of variation of flow

Lamivudine

S. No.	Flow rate (ml/min)	System suitability results	
		USP plate count	USP tailing
1	0.7	8564.30	1.21
2	0.8	7360.35	1.22
3	0.9	8521.25	1.25

Zidovudine

S. No.	Flow rate (ml/min)	System suitability results	
		USP plate count	USP tailing
1	0.7	8567.20	1.25
2	0.8	7630.10	1.29
3	0.9	8694.36	1.24

Results for actual mobile phase composition (40:60 Water: Methanol) have been considered from accuracy standard.

Acceptance criteria

A tailing factor not more than 2, Plate count not less than 2000.

Summary and conclusion

The reliability and suitability of the method would be seen from recovery studies, and the further studies indicate that there is no interference due to excipients.

✓ System suitability parameters were calculated which includes efficiency, plate count, tailing factor and % RSD

✓ The precision of the method was studied by making repeated injections of the samples and system precision values were determined.

✓ The method was validated for linearity, accuracy, precision and robustness.

✓ The method is simple, specific & easy to perform and requires a short time to analyze the samples.

✓ The method was found to have accurate, precise and robust.

✓ Hence, it was concluded that the RP-HPLC method developed was very much suitable for routine analysis for Lamivudine and Zidovudine in bulk.

CONFLICT OF INTERESTS

Declare none

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