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# COST EFFECTIVE AND SIMPLE METHOD FOR ROUTINE ANALYSIS OF ZIDOVUDINE AND LAMIVUDINE IN TABLET DOSAGE FORM BY RP - HPLC

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## ABSTRACT

The objective of the current study is to develop a validated RP- HPLC method for the simultaneous estimation of Zidovudine[1] and Lamivudine[2] in tablet dosage form. The method follows sample preparation step followed by separation on Phenomenex C18 column (250 x 4.6mm id, 5 µm particle size utilizing Shimadzu HPLC (LC-10AT VP) equipped with UV-Visible and PDA detector. The mobile phase was prepared by mixing methanol, acetonitrile and water in the ratio of 60:20:20v/v/v. The eluted peaks were detected by photo diode array (PDA) detector at a wavelength of 265nm. The method was validated with respect to linearity, accuracy, precision, and robustness. The utility of the procedure was verified by its application to formulations and it was found that the developed method is fast, accurate, precise, selective, reproducible and cost effective and can be used for the routine laboratory analysis of tablet dosage form in institutions and industries.

**KEYWORDS: Zidovudine and Lamivudine, NRTI, RP-HPLC, simultaneous estimation, method development and validation, pharmaceutical dosage form**

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# INTRODUCTION

Many serious diseases such as ebola, avian influenza, AIDS and SARS are caused by viruses. Other diseases are under investigation as to whether they too have a virus as the causative agent, such as the possible connection between human herpes virus six (HHV6) and neurological diseases such as multiple sclerosis and chronic fatigue syndrome. Because viruses use vital metabolic pathways within host cells to replicate, they are difficult to eliminate without using drugs that cause toxic effects to host cells in general. The most effective medical approaches to viral diseases are vaccinations to provide immunity to infection, and antiviral drugs that selectively interfere with viral replication. The life cycle of HIV varies to a great extent since its entry into the cell. HIV lacks the proofreading enzymes to correct errors made when it converts its RNA into DNA via reverse transcription. Its short life-cycle and high error rate cause the virus to mutate very rapidly, resulting in a high genetic variability of HIV. Most of the mutations either are inferior to the parent virus (often lacking the ability to reproduce at all) or convey no advantage, but some of them have a natural selection superiority to their parent and can enable them to slip past defenses such as the human immune system and antiretroviral drugs. The more active copies of the virus the greater the possibility of resistance exhibited by them towards the therapy. Combinations of antiretrovirals create multiple obstacles to HIV replication to keep the number of offspring low and reduce the possibility of a superior mutation. As a result, the standard of care is to use combinations of antiretroviral drugs[3]. In recent years, drug companies have worked together to combine these complex regimens into simpler formulas, termed fixed-dose combinations. The combination of two NRTIs such as Zidovudine (AZT) and Lamivudine (3TC) is effective in HIV therapy. Zidovudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). It is a structural analog of thymidine, inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA.

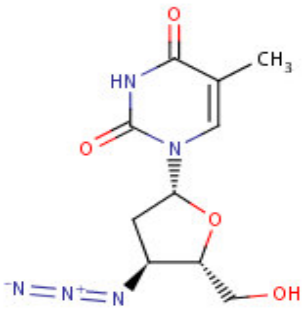


**Figure 1**
***Zidovudine***

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B virus (HBV) and it is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.

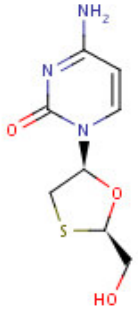


**Figure 2**
***Lamivudine***

Literature survey reveals that the drug combination is proposed for authorized pending monograph[4]. The reported methods for simultaneous estimation of Zidovudine and Lamivudine are mainly developed keeping in view the requirements of research mainly in biological fluids using high end analytical instruments like HPLC – MS, ion pair chromatography, fluorimetric detection using extraction procedures for sample preparation. Consequently, certain methods are also focused on the analysis of the drug from biological fluids [5-10]. The aim of this research work is to develop a simple and cost effective HPLC method for simultaneous estimation of AZT and 3TC antiretroviral fixed dose combinations (FDCs) without the necessity of any kind of sample pre-treatment. This paper describes the development and validation of RP-HPLC method, using PDA detector, for the simultaneous estimation of AZT and 3TC in fixed dose combination tablets. The method has industrial suitability for the routine analysis of the formulation due to its rapidity, ease, economic viability, sensitivity, selectivity and lack of excipients' interference.

## *EXPERIMENTAL*

### *Apparatus*

Chromatographic analysis was performed with a Shimadzu HPLC (LC-10AT VP) equipped with UV-Visible and PDA detector. Software – Class VP was used for LC peak integration along with data acquisition and data processing. The column used was Phenomenex C18 column (250 x 4.6mm id, 5 µm particle size). Injector was manual injector loop with injection volume of 20 µl. Ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai) was used for sonication for degassing of mobile phase. UV-1700, Shimadzu, Japan with UV Probe 2.0 software was used to obtain the overlay spectra of the drugs to determine the analytical wavelength. The standard samples were obtained as gift samples from Emcure Pharma Ltd., Pune, along with their certificate of analyses. The standard substances were weighed on Sartorius CP224S analytical balance (Gottingen, Germany).

## REAGENTS AND MATERIALS

The combination product was obtained as tablets from local pharmacy. HPLC grade acetonitrile used were purchased from S.D. Fine chemicals, Mumbai. Water HPLC grade from Finar reagents, Ahmedabad, was used for the preparation of mobile phase. Whatman filter paper no. 41 was used as a filter medium procured from S.D. Fine chemicals, Mumbai.

### *Preparation of mobile phase*

HPLC grade solvents were used in separate bottle of gradient pumps as mobile phase. A mixture of methanol, acetonitrile and water in the ratio of 60:20:20 v/v/v adjusted by gradient pump operated by LC solution software. Mixed solvents were filtered through nylon 0.45 µm membrane filter and degassed by the instrument and used as mobile phase.

### *Preparation of standard solutions*

### *Preparation of standard stock solution (100 µg/ml)*

Accurately weighed 10 mg of Lamivudine was transferred to 100 ml volumetric flask, dissolved

in 50 ml methanol and diluted up to mark with methanol to prepare standard stock solution of Lamivudine having concentration 100µg/ml. Accurately weighed 10 mg of Zidovudine was transferred to 100 ml volumetric flask, dissolved in 50 ml methanol and diluted up to mark with methanol to prepare standard stock solution of Zidovudine having concentration 100µg/ml.

### *Preparation of working standard solution*

From the standard stock solution of Lamivudine (100µg/ml) aliquots of 0.05, 0.2, 0.5, 1.0, 1.2 and 1.5 ml were taken in separate volumetric flasks and diluted with methanol to get concentrations in the range of 0.5 – 15 µg/ml. Similarly from the above stock solution of Zidovudine appropriate aliquots of 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 ml were taken in separate volumetric flasks and diluted with methanol to get concentrations in the range of 1 - 25 µg/ml.

### *Preparation of sample solution*

COMBIVIR (Lamivudine 150mg and Zidovudine 300mg) tablets were used for analysis. Twenty tablets were powdered and an accurate quantity of tablet powder equivalent to the 10mg of Lamivudine and 20 mg of Zidovudine was weighed and transferred to 100 ml volumetric flask, dissolved in methanol (60 ml) and sonicated for 30 min. The solution was filtered through Whatman filter paper No. 41 and residue was washed with methanol. The solution was diluted up to the mark with methanol.

### *Determination of wavelength of maximum absorbance*

The standard solutions of both Lamivudine (5µg/ml) and Zidovudine (5µg/ml) were injected in the system and spectrum in the range of 200 to 400 nm was recorded.

### *Chromatographic conditions*

| S.no. | Parameter | Description / Value |
|---|---|---|
| 1. | Stationary Phase | Phenomenex® $C_{18}$ column with 250 mm x 4.6 mm i.d and 5 µm particle size |
| 2. | Mobile Phase | A mixture of methanol, acetonitrile and water in the ratio of 60:20: 20 v/v/v |
| 3. | Flow Rate | 1.0 ml/min |
| 4. | Detection wavelength | 265 nm |
| 5. | Detector | Photodiode array (PDA) detector |
| 6. | Injector | Manual injector loop |
| 7. | Injection volume | 20µl |
| 8. | Column Temperature | Ambient |
| 9. | Run Time | 8.5 min |
| 10 | Diluent | Methanol |

**Table 1**

***Chromatographic Conditions for Simultaneous Estimation of Lamivudine and Zidovudine***

### *Method validation*

The method was validated with respect to the following parameters given below as per ICH guidelines [11].

### *Linearity and range*

Accurately measured working standard solutions of Lamivudine (0.5, 2, 5, 10, 12 and 15 ml) and Zidovudine (1, 5, 10, 15, 20 and 25 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. 20 µl of each of these solutions was injected using manual injector loop under the final chromatographic conditions described above. Calibration curve was constructed by plotting peak area versus concentration of Lamivudine and Zidovudine and the regression equation was calculated.

### *Accuracy (% Recovery)*

The accuracy of the method was determined by calculating recoveries of Lamivudine and Zidovudine by the standard addition method. For this known amounts of standard solutions of Lamivudine and Zidovudine (50, 100, and 150 % level) were added to preanalyzed sample solutions. The amount of Lamivudine and Zidovudine was analyzed by using the regression equation of the calibration curve.

### *Precision*

### *Method precision (% Repeatability)*

The precision of the instrument was checked by repeatedly injecting (n = 6) solution of Lamivudine (5 µg/ml) and Zidovudine (10 µg/ml).

The results were reported in term of % coefficient of variance (% CV) should not more than 2 %.

***Intermediate precision***

Intermediate precision was evaluated in terms of intraday and interday precision. The intraday precision was investigated by analysing three different solutions of Lamivudine (4.0, 8.0 and 12 µg/ml) and Zidovudine (8.0, 12.0 and 24.0 µg/ml). The interday precision was investigated by analysing three different standard solutions of Lamivudine (4.0, 8.0 and 12.0 µg/ml) and Zidovudine (8.0, 12.0 and 24.0 µg/ml) on different days. The results were reported in terms of % coefficient of variance (% CV).

***Robustness***

Method robustness was performed by applying small changes in the composition of mobile phase, detection wavelength and flow rate. Robustness of the method was done at three different concentration levels of Lamivudine (4.0, 8.0 and 12.0 µg/ml) and Zidovudine (8.0, 12.0 and 24.0 µg/ml). The results were expressed in terms of % CV.

***Limit of detection and quantification***

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by using the following equations:

$$LOD = 3.3\ \sigma / S$$

And

$$LOQ = 10\ \sigma / S$$

Where σ = standard deviation of the response and S = slope of the calibration curve

***Specificity and selectivity***

The specificity of the method was established through resolution factor of the drug peak from the nearest resolving peak and also among all other peaks. Selectivity was confirmed through peak purity data using a PDA detector. To assess the method specificity, tablet powder without Lamivudine and Zidovudine (placebo) was prepared with the excipients as required for commercial preparation and compared with respective drug standard to evaluate specificity of the method. Representative chromatograms of placebo and standard were compared for retention time, resolution factor and purity.

***System suitability***

The system suitability parameters like theoretical plates ($T_p$), and asymmetry factor (As), capacity factor (K'), resolution ($R_s$), retention time (RT) and tailing factor ($T_f$) reported in European Pharmacopoeia were calculated by Class VP LC solution software. The HPLC system was equilibrated with the initial mobile phase composition, followed by six injections of the standard solutions having the same concentration. These six consecutive injections were used to evaluate the system suitability on each day of method validation. In order to establish system suitability for the instrument, six consecutive injections of Lamivudine and Zidovudine was prepared from working standard solution and analyzed.

***Solution stability***

The solution stability of Lamivudine and Zidovudine in the proposed method was carried out by leaving both the test and standard solution in tightly capped volumetric flask at room temperature for 24 hours. The same sample solutions were assayed for interval of 6 hours up to the 24 hours throughout the study period. The obtained results were compared with the freshly prepared solution.

***Assay procedure***

Drug contents were calculated by comparison with the appropriate standard solution of the drug. No interferences due to excipients were detected in the chromatograms produced.

## RESULTS AND DISCUSSION

***Determination of analytical wavelength***

Solutions of Lamivudine (5µg/ml) and Zidovudine (5µg/ml) were injected in the system and scanned between 200 and 400 nm with the help of PDA detector. The UV overlay spectra of both the drugs are shown in Figure 3.

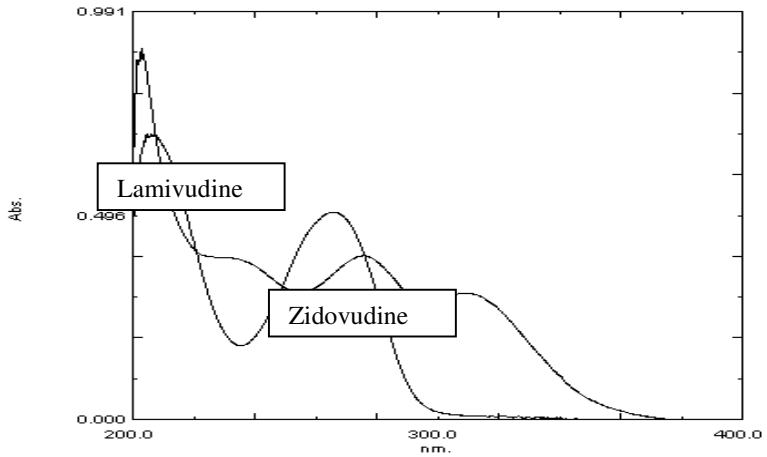


**Figure 3**
***Overlay spectra of Lamivudine and Zidovudine***

From the UV spectra it was observed that maximum response was obtained at 265nm. So, 265nm was selected as an analytical wavelength.

***Method optimization***

The main aim of the method is to resolve the drugs from excipients found in formulation, $C_{18}$ column (250 mm x 4.6 mm i.d., 5 µm particle size) was preferred over other reverse phase columns. A Phenomenex $C_{18}$ was preferred as it has a high carbon loading with very closely packed material to give high performance over other $C_{18}$ columns. Initially different mobile phases have been tried. The selected mobile phase was then passed with different flow rate, but the best result was obtained at a flow rate of 1ml/min. An acceptable resolution with reasonable peak shapes and peak purity were achieved by using a mixture of methanol, acetonitrile and water in the ratio of 60:20:20v/v/v with a flow rate of 1 ml/min at wavelength maxima of 265nm. The method parameters were optimized for the analysis of Lamivudine and Zidovudine in tablet dosage form. A representative chromatogram is shown in Figure 4 and 5, which satisfies all the system suitability criteria, better resolution of the peak from the solvent peak with clear base line separation.

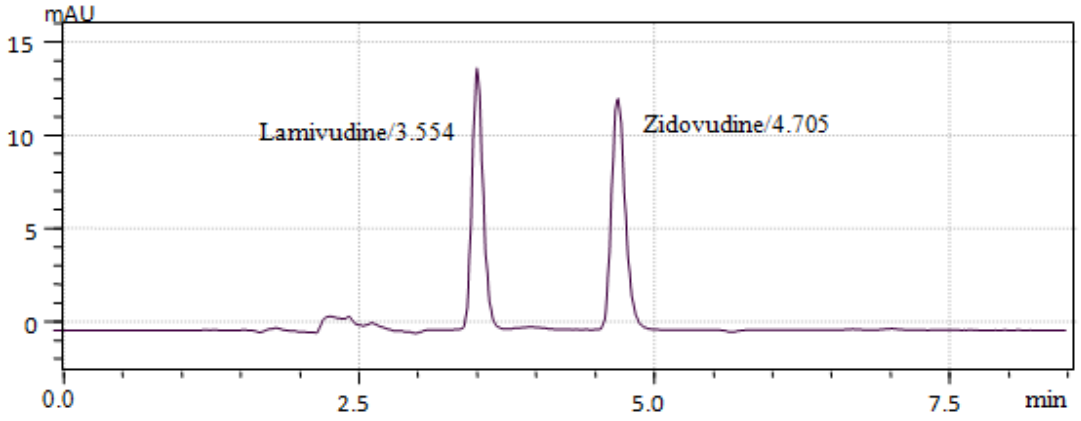


**Figure 4**
***Lamivudine (5 µg/ml) and Zidovudine (10 µg/ml) chromatogram in formulation at 265 nm***

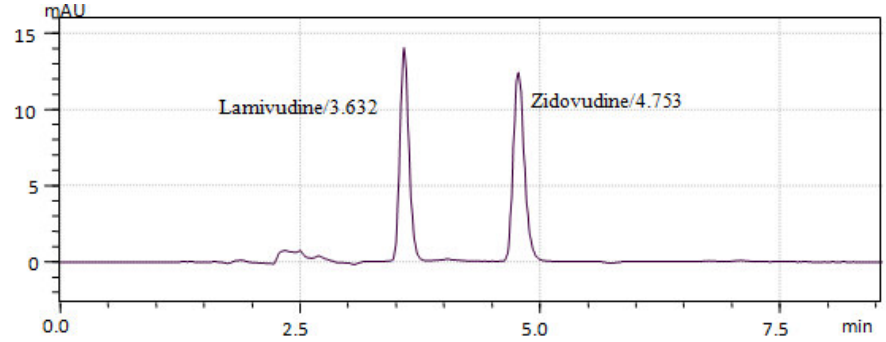


**Figure 5**
***Lamivudine (5 µg/ml) and Zidovudine (10 µg/ml) chromatogram at 265 nm***

***VALIDATION OF THE PROPOSED METHOD***

The proposed method was validated for various parameters like system suitability, specificity, linearity, precision, accuracy, LOQ and LOD.

***Linearity***

Linearity of the method was evaluated at six concentration levels by diluting the working standard solution in the concentration range from 0.5 - 15 µg/ml for Lamivudine and 1 – 25 µg/ml for Zidovudine. The results show that an excellent correlation existed between the peak area and concentration of analyte. The calibration curve was prepared by plotting the peak area versus the concentration and regression equation was calculated. The calibration curve was repeated for five times so the average results are mentioned in table 2. The calibration curves are shown in figures 6 and 7. The optical and regression characteristics for analysis of Stavudine and Lamivudine by RP-HPLC method are mentioned in table 3.

| Conc of Lamivudine µg/ml | Peak area | Conc of Zidovudine µg/ml | Peak area |
|---|---|---|---|
| 0.5 | 2756 | 1 | 4218 |
| 2 | 21756 | 5 | 25364 |
| 5 | 61458 | 10 | 67352 |
| 10 | 130452 | 15 | 101364 |
| 12 | 153320 | 20 | 141645 |
| 15 | 180756 | 25 | 184734 |

**Table 2**
***Data for Calibration Curve for Lamivudine and Zidovudine***

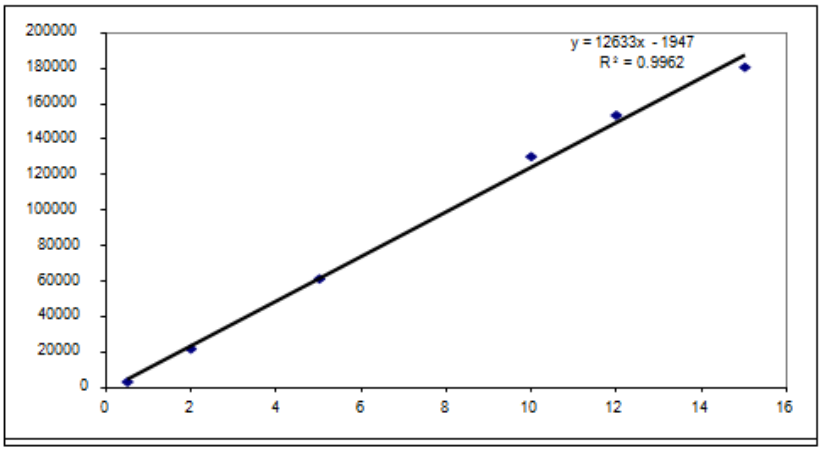


**Figure 6**
***Calibration curve for Lamivudine for simultaneous estimation with Zidovudine***

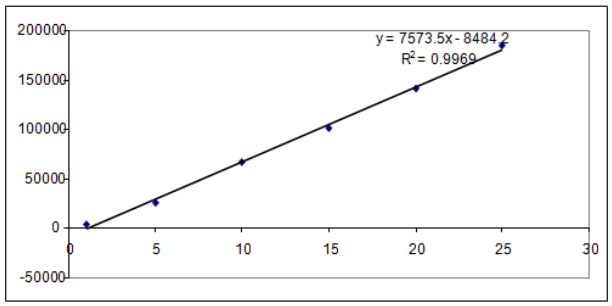


**Figure 7**
***Calibration Curve for Zidovudine for Simultaneous Estimation with Lamivudine***

| Parameters | Lamivudine | Zidovudine |
|---|---|---|
| Concentration range (µg/ml) | 0.5 - 15 | 1 - 25 |
| Limit of Detection (LOD) (µg/ml) | $4.9 \times 10^{-5}$ | $9.8 \times 10^{-5}$ |
| Limit of Quantification (LOQ) (µg/ml) | $1.5 \times 10^{-4}$ | $2.9 \times 10^{-4}$ |
| Regression equation (y*= a + bc) | | |
| Slope (b) | 12633 | 7573.5 |
| Intercept (a) | –1947 | –8484.2 |
| Correlation coefficient (r) | 0.9962 | 0.9969 |
| y*= a + bc, where c is the concentration | | |

**Table 3**
***Optical and Regression Characteristics for Analysis of Stavudine and Lamivudine***

***Precision***
***Method precision***
The results of repeatability (method precision) experiment are shown in Table 4. Method precision was determined by repeatedly injecting 5.0 µg/ml concentration of Lamivudine and 10 µg/ml of Zidovudine (n = 6). The developed method was found to be precise as the % CV values for repeatability study was < 1.0 %.

| Drug | Concentration | RT | %CV | Area |
|---|---|---|---|---|
| **Lamivudine** | 5.0 µg/ml | 3.586 | 0.574 | 59682 ± 0.486 |
| **Zidovudine** | 10 µg/ml | 4.722 | 0.503 | 59620 ± 0.613 |

**Table 4**
***Method precision data of Lamivudine and Zidovudine by RP-HPLC method***

***Intermediate precision***
The results of intermediate precision experiment for both intraday and interday are shown in Table 5. Replicate analyses of three concentrations of the standard solution show good reproducibility. The developed method was found to be precise as the % CV values for intermediate precision study was < 2.0 %.

| Lamivudine µg/ml | Intra-day measured mean area, % CV (n=6) | Lamivudine µg/ml | Inter-day measured mean area, % CV (n=6) |
|---|---|---|---|
| 4 | 47685 ± 1.201 | 4 | 48021 ± 1.437 |
| 8 | 102164 ± 1.313 | 8 | 995340 ± 1.503 |
| 12 | 148326± 1.034 | 12 | 146857 ± 1.551 |
| **Zidovudine µg/ml** | **Intra-day measured mean area ± S.D, % CV (n=6)** | **Zidovudine µg/ml** | **Inter-day measured mean area ± S.D, % CV (n=6)** |
| 8 | 45032.021 ± 1.261 | 8 | 44857.394 ± 1.775 |
| 12 | 68967.031 ± 1.119 | 12 | 69342.24 ± 1.194 |
| 24 | 142496.385 ± 1.302 | 24 | 143293.152 ± 1.573 |

***% CV = Coefficient of variance***
***n = Number of replicate***

**Table 5**
***Intermediate precision data of Lamivudine and Zidovudine***
***RP-HPLC method Accuracy (% Recovery)***

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of drug (50, 100, and 150 %) was added to the pre analyzed sample solution. This solution was analyzed under the chromatographic conditions as mentioned in above section. The assay was repeated over 3 consecutive days to obtain intermediate precision data. The results of accuracy are shown in Table 6.

| Drug | Known conc. µg/ml | Added conc. µg/ml | AUC ± SD, %CV | % Recovery |
|---|---|---|---|---|
| **Lamivudine** | 4 | 2 (50%) | 71672.217 ± 1.732, 1.759 | 97.0495 |
| | 4 | 4 (100%) | 97804.032 ± 1.384, 1.395 | 98.6753 |
| | 4 | 6 (150%) | 122385.021 ± 1.687, 1.696 | 98.3930 |
| **Zidovudine** | 8 | 4 (50%) | 68393.03 ± 1.405, 1.432 | 96.4165 |
| | 8 | 8 (100%) | 94284.05 ± 1.328, 1.367 | 98.6575 |
| | 8 | 12 (150%) | 117945 ± 1.574, 1.584 | 98.1247 |

***S.D = Standard déviation***
***% CV = Coefficient of variance***
***n = Number of replicate***

**Table 6**
***Accuracy (% Recovery) study of Lamivudine and Zidovudine by RP-HPLC method***

### *Robustness*

To evaluate the robustness of the proposed method, experimental conditions were deliberately altered and the response of the drugs was recorded. The results of minor changes in composition of mobile phase, wavelength and flow rate are shown in Table 7.

| Parameter | Modification | % Recovery ± S.D, % CV (n=6) Lamivudine | % Recovery ± S.D, % CV (n=6) Zidovudine |
|---|---|---|---|
| Flow rate (1 ml/min) | ± 0.1 | 98.548 ± 1.234, 1.346 | 98.854 ± 1.623, 1.697 |
| Mobile phase composition | 61:18:21 | 98.347 ± 1.596, 1.617 | 98.342 ± 1.365, 1.379 |
| methanol: acetonitrile : water (60:20:20v/v/v) | 59:22:19 | 99.134 ± 1.648, 1.692 | 98.684 ± 1.165, 1.197 |
| Wave length (265 nm) | ± 1 | 99.597 ± 1.692, 1.724 | 99.424 ± 1.276, 1.296 |

***S.D = Standard déviation***
***% CV = Coefficient of variance***
***n = Number of replicate***

**Table 7**
***Intra-day robustness data of Lamivudine and Zidovudine by RP-HPLC method***

### *Limit of detection and quantitation*

These data show that the method is sensitive for the determination of Lamivudine and Zidovudine. The results are given in table 8.

| | Standard Deviation (σ) | Slope of cal. curve | LOD µg/ml | LOQ µg/ml |
|---|---|---|---|---|
| **Lamivudine (8µg/ml)** | 0.1868 | 12633 | 0.000049 | 0.00015 |
| **Zidovudine (20µg/ml)** | 0.2237 | 7573.5 | 0.000098 | 0.00029 |

**Table 8**
***LOD and LOQ for Lamivudine and Zidovudine***

### Specificity and selectivity

The resolution factor for Lamivudine and Zidovudine from the nearest resolving solvent peak was > 3 in all samples. The placebo shows no detector response near retention times of 3.561 min and 4.694 min, while the Lamivudine and Zidovudine standards display good resoluted peak (Figure 8) and no interference from excipients present in the formulation (Figure 9) indicates specific nature of the method. The purity curves and data (Figures 10 and 11 & Table 9) of Lamivudine and Zidovudine show that no other excipients are co-eluted with the drug and the peaks are pure in nature.

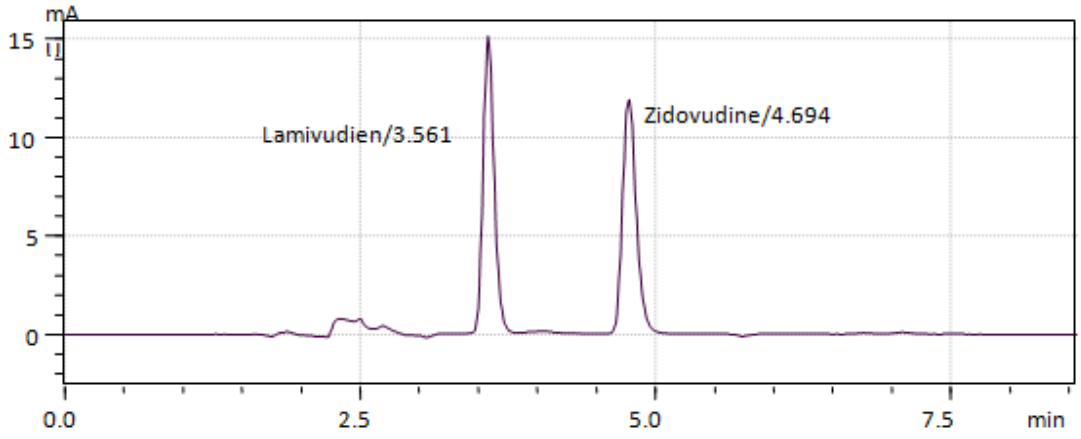


**Figure 8**
***Lamivudine and Zidovudine chromatogram in formulation at 265 nm***

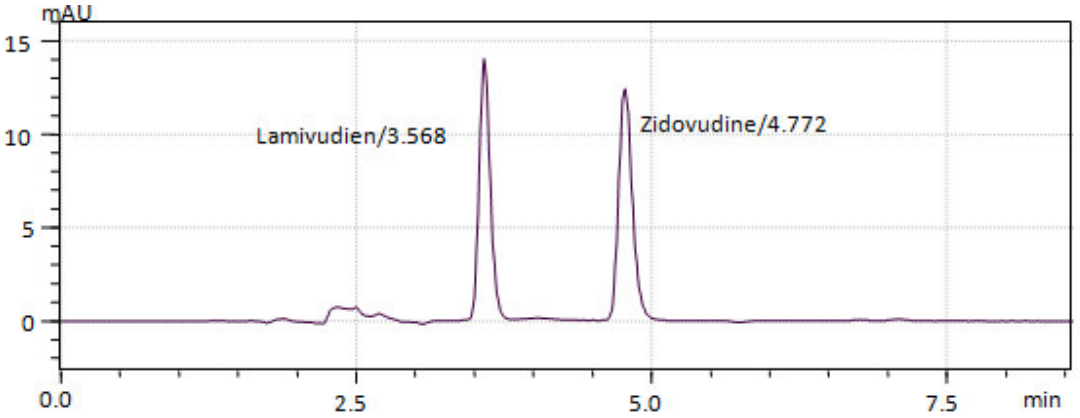


**Figure 9**
***Lamivudine and Zidovudine chromatogram at 265 nm***

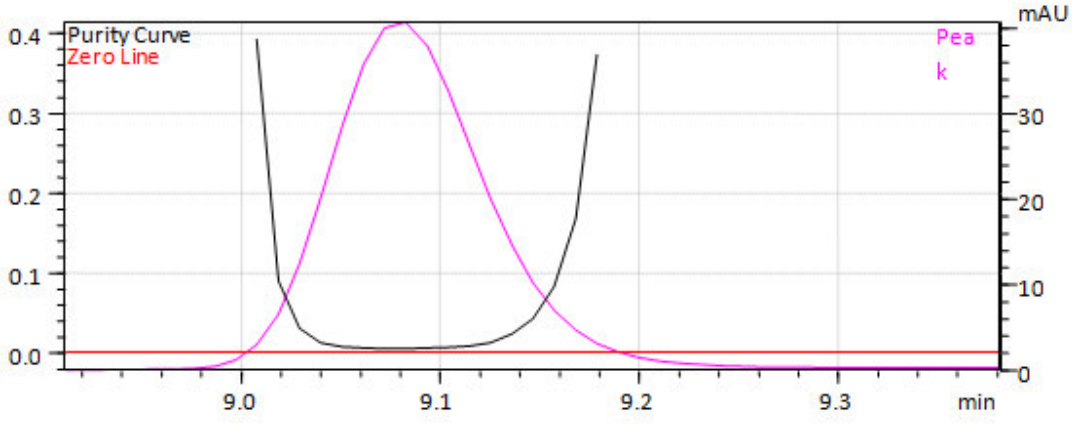


**Figure 10**
***Peak Purity plot of Lamivudine with purity 0.99999***

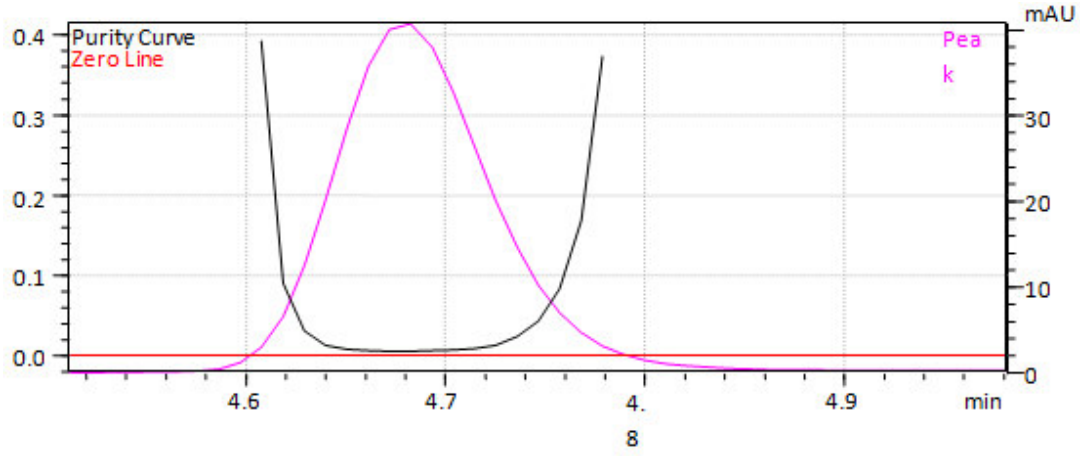


**Figure 11**
***Peak Purity plot of Zidovudine with purity 0.99999***

| Drug | Peak purity Index | Single point Threshold | Minimum peak purity threshold |
|---|---|---|---|
| Lamivudine | 0.99999 | 0.99968 | 3136 |
| Zidovudine | 0.99999 | 0.99956 | 4865 |

**Table 9**
***Table showing Peak purity data***

***System suitability***

As system suitability test was an integral part of chromatographic method development and were used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Lamivudine and Zidovudine were evaluated. The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values. The obtained parameters are given in table 10 and they are found in between the acceptance criteria.

| Parameter | Value for Lamivudine | Value for Zidovudine |
|---|---|---|
| Retention time (Minutes) | 3.568 | 4.772 |
| Resolution (Rs) | 3.84 | 3.84 |
| Theoretical plates ($T_P$) | 4975 | 3057 |
| Tailing factor ($T_f$) | 0.51 | 1.25 |
| Asymmetric factor ($A_f$) | 1.04 | 1.13 |
| Capacity factor (K') | 3.67 | 5.41 |

**Table 10**
***System suitability parameters for Lamivudine and Zidovudine***

***Solution stability***

The % CV of the assay of Lamivudine and Zidovudine during solution stability experiments were within 2%. No significant changes were observed in the content of standard drug solution during solution stability and mobile phase stability experiments when performed using the method. The solution stability and mobile phase stability experiment data confirms that the sample and standard in solvent and mobile phases used during assay determination were stable for at least 24 hours. The results of the solution stability data are given in table 11.

| Drug and concentration → | Lamivudine 5µg/ml | | Zidovudine 5µg/ml | |
|---|---|---|---|---|
| | Time (Hr) | Peak area | Time | Peak area |
| | 06 | 61353 | 06 | 25234 |
| | 12 | 61132 | 12 | 25118 |
| | 18 | 60877 | 18 | 24998 |
| | 24 | 60236 | 24 | 24912 |

**Table 11**
***Data for solution stability***

## CONCLUSION

A validated RP-HPLC analytical method has been developed for the simultaneous estimation of Lamivudine and Zidovudine in bulk and in dosage form. The proposed method is simple, accurate, precise, specific, and has ability to separate drug from excipients in the formulation. The method is suitable for routine simultaneous analysis of Lamivudine and Zidovudine in tablet formulation. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS. The prime importance was given to develop a fast and simple RP-HPLC method. The method requires no sample pre treatment and is quite economical for routine analyses. The proposed method meets the system suitability criteria, peak integrity and resolution for the drugs. The detection and quantification limits show that the method is very sensitive. High recoveries and acceptable % CV values confirm that the proposed method is accurate and precise. The analytical results demonstrate the ability of the developed method to assay both the drugs in the presence of their excipients. Assay results found from the study show that the method can successfully applied for the simultaneous estimation of Lamivudine and Zidovudine in formulation. Hence, the method is recommended for routine quality control analysis of both the drugs in combination in the tablet formulation.

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