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ORIGINAL ARTICLE

# Development and validation of a normal-phase HPTLC method for the simultaneous analysis of Lamivudine and Zidovudine in fixed-dose combination tablets

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**Abstract** Simultaneous quantification of Lamivudine and Zidovudine in tablets by HPTLC method was developed and validated. The chromatograms were developed using a mobile phase of toluene:ethyl acetate:methanol (4:4:2, v/v/v) on pre-coated plate of silica gel GF aluminum TLC plate and quantified by densitometric absorbance mode at 276 nm. The $R_f$ values were $0.41 \pm 0.03$ and $0.60 \pm 0.04$ for Lamivudine and Zidovudine, respectively. The linearity of the method was found to be within the concentration range of 50−250 ng/spot for Lamivudine and for Zidovudine, it was 100−500 ng/spot. The lower limits of detection and quantification were 2.23 ng/spot and 7.90 ng/spot for Lamivudine and 2.90 ng/spot and 8.85 ng/spot for Zidovudine. The method was also validated for precision, specificity and recovery. This developed method was used to analyze fixed-dose tablets (Duovir, Cipla Ltd) samples of Lamivudine and Zidovudine.

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## 1. Introduction

Lamivudine (4-amino-1-[(2$R$,5$S$)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2dihydro pyrimidine-2-one) (Fig. 1A) and Zidovudine (1-[(2$R$,4$S$,5$S$)-4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione) (Fig. 1B) were anti-HIV drugs (reverse transcriptase inhibitors). Lamivudine and Zidovudine have been reported to be quantified individually or in combination with other drugs [1–3]. The literature survey reveals that there are analytical methods available for determination of Lamivudine and Zidovudine from biological matrices, bulk drug and dosage forms, and for determination of Lamivudine and Zidovudine with combination of other antiviral drugs by RP-HPLC/MS [4–7].
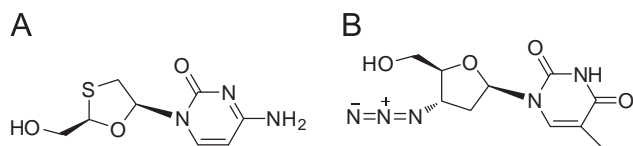
A

B



**Figure 1** (A) Lamivudine and (B) Zidovudine.

Habte et al. [8] had reported HPTLC method for the analysis of Lamivudine and Zidovudine using the solvent system of toluene/chloroform/methanol (1:6:3, v/v/v) with the lower limit of detection and quantification of 3.06 ng/spot and 9.28 ng/spot for Lamivudine and 3.34 ng/spot and 10.13 ng/spot for Zidovudine. In this study, we report the HPTLC method for the analysis of Lamivudine and Zidovudine using a solvent system of toluene:ethyl acetate:methanol (4:4:2, v/v/v), the lower limits of detection and quantification are 2.23 ng/spot and 7.90 ng/spot for Lamivudine and 2.98 ng/spot and 8.85 ng/spot for Zidovudine.

## 2. Experimental

### 2.1. Chemicals and reagents

Pure Lamivudine powder and Zidovudine powder were kind gifts from Micro Labs, Bangalore, India. Commercial tablets (Duovir, Cipla Ltd) containing Lamivudine (150 mg) and Zidovudine (300 mg) were used for the study. Toluene, ethyl acetate and methanol used were of analytical grade (E. Merck, Mumbai, India). All the other chemicals used were also of analytical grade (E. Merck, India).

### 2.2. Instrumentation and conditions

HPTLC plates pre-coated with silica gel GF aluminum TLC plate, (10 cm 10 cm) were from Merck. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with a win-CATS 1.4.0 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG LINOMAT V under nitrogen gas ow, and developed in a CAMAG 10 cm 10 cm twin trough chambers.

### 2.3. Standard preparation

Lamivudine and Zidovudine (10 mg each) were accurately weighed and transferred into 10 mL volumetric asks, and dissolved in 10 mL of methanol. Both solutions were further diluted with methanol to get the final concentration of 2 µg/mL. The stock solutions were further diluted with methanol to obtain a working standard solution with final concentrations of 50, 100, 150, 200 and 250 ng/mL for Lamivudine and 100, 200, 300, 400 and 500 ng/mL for Zidovudine respectively, which were used for calibration purposes.

### 2.4. Preparation of sample solution

For analysis of tablet dosage form, twenty tablets, each containing 150 mg of Lamivudine and 300 mg Zidovudine, were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 10 mg Lamivudine and Zidovudine was accurately weighed and dissolved in 10 mL of methanol. The solution was sonicated for 30 min, filtered through the Whattman No. 41 filter paper and the residue was washed with methanol. The volume of the filtrate was adjusted to 10 mL with the same solvent. This solution was further diluted with methanol to get the same concentration as that of the final standard solution.

### 2.5. Chromatographic conditions

Lamivudine and Zidovudine reference standard solutions were prepared using methanol as solvent. Solutions of 2 µL were applied to the HPTLC plates as spot bands of 6 mm using LINOMAT V. Application positions were at least 15 mm from the sides and 10 mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left for saturation with mobile phase vapor for 10 min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7 cm. Then the plates were dried on a hot plate. All the analyses were carried out in a laboratory with temperature control (20–24 °C).

Densitometry scanning was done in absorbance mode at 276 nm using a deuterium lamp. The slit dimensions were set at 6 mm 0.30 mm, the scanning speed of 10 mm/s, and the data resolution at 100 µm/step. Single wavelength detection was performed since the main components were only analyzed.

### 2.6. Method validation

The developed method was validated as per the International Conference on Harmonization (ICH) [9,10] guidelines with respect to linearity and range, specificity, precision, accuracy, limit of detection and limit of quantification.

## 3. Results and discussion

### 3.1. Linearity and range

A stock standard solution was prepared for both Lamivudine and Zidovudine; they were serially diluted to yield five standard solutions. A volume of 2 µL of each solution was applied to the HPTLC plate to deliver 50, 100, 150, 200 and 250 ng of Lamivudine per spot, 100, 200, 300, 400 and 500 ng of Zidovudine per spot. This was done in triplicate and repeated for three days. For each concentration, the applied spot bands were evenly distributed across the plate to minimize possible variation along the silica layer. The results are indicated in Table 1.

### 3.2. Precision

The repeatability (intra-days precision) is expressed as percentage relative standard deviations (% RSD) for the Lamivudine at the concentrations of 50, 100 and 150 ng/spot, their % RSD values were 0.48, 0.52 and 0.46, respectively, and for the time-different intermediate precision (inter-days precision) the % RSD values were 0.38, 0.62 and 0.39, respectively. The % RSD values of intra-days precision for Zidovudine at the concentrations of 100, 200 and 300 ng/spot were 0.34, 0.45, 0.68, and for inter-days precision the % RSD levels were 0.43, 0.82 and 0.52, respectively. The pooled repeatability precisions

**Table 1** Linearity results.

| Components | Concentration range (ng/spot) | Equation for regression line | $r^2$ |
|---|---|---|---|
| Lamivudine | 50–250 | $Y=2319.86x+22.69$ | 0.9991 |
| Zidovudine | 100–500 | $Y=3843.13x+18.92$ | 0.9995 |

**Table 2** Accuracy and intermediate precision.

| Drugs | Concentration (ng/spot) | Intra-day | | Inter-day | |
|---|---|---|---|---|---|
| | | Accuracy (%)[a] | Precision (% RSD) | Accuracy (%)[a] | Precision (% RSD) |
| Lamivudine | 50 | 98.57±1.27 | 0.48 | 98.84±1.34 | 0.38 |
| | 100 | 99.83±1.24 | 0.52 | 99.74±1.52 | 0.62 |
| | 150 | 99.58±1.32 | 0.46 | 99.28±1.27 | 0.39 |
| Zidovudine | 100 | 99.26±1.63 | 0.34 | 99.05±1.12 | 0.43 |
| | 200 | 98.69±1.52 | 0.45 | 99.21±1.36 | 0.82 |
| | 300 | 99.34±1.34 | 0.68 | 99.28±1.27 | 0.52 |

[a]Mean of three replicate.

were 0.49 and 0.56 for Lamivudine and Zidovudine concentrations, respectively, and the pooled time-different intermediate precisions were 0.50 and 0.59, respectively. The % RSD levels of intra-day and inter-day precision were less than 1.0 in all cases, which indicated that there were no significant variations in the analysis of Lamivudine and Zidovudine at the concentrations, which are shown in Table 2.

### 3.3. Accuracy

The accuracy was assessed by the methodological recovery. The recovery of the method was calculated by comparing the determined concentration of spiked samples to the theoretical concentrations. The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. The intra-day and inter-day percentages of accuracy obtained for Lamivudine at the concentrations of 50, 100, 150 ng/spot, and for Zidovudine at the concentrations of 100, 200 and 300 ng/spot, are respectively shown in Table 2. The % recoveries of intra-day for Lamivudine were 98.57±1.27%, 99.83±1.24% and 99.58±1.32%, respectively, the mean recovery for all the concentration levels was 99.26±1.72%.

For Zidovudine, the % recoveries of intra-day were 99.26± 1.63%, 98.69±1.52% and 99.34±1.34%, respectively (Table 2). The mean value covering all the concentration levels was 99.09±1.46%.

### 3.4. Limits of detection and quanti cation

The limit of detection was found to be 2.23 ng/spot and 7.90 ng/spot for Lamivudine and Zidovudine, respectively. The limit of quantification was found to be 2.90 ng/spot and 8.85 ng/spot for Lamivudine and Zidovudine, respectively, which was lower than that reported earlier [8,11].
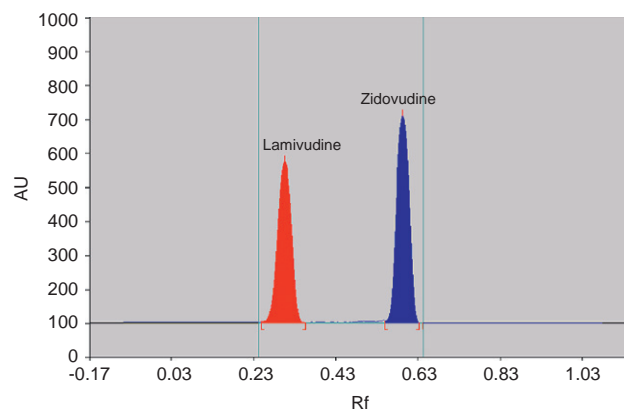


**Figure 2** Chromatogram of fixed-dose tablet (Duovir, Cipla) solution of Lamivudine and Zidovudine. Mobile phase—toluene:ethyl acetate:methanol (4:4:2, v/v/v), detection at 276 nm.

### 3.5. Speci city

The chromatogram of the solution, which was not spiked with Lamivudine and Zidovudine, did not show any spot, while the chromatogram of the solution of the tablet matrix spiked with Lamivudine and Zidovudine showed clear, compact and well-separated peaks of Lamivudine and Zidovudine (Fig. 2). Moreover, from Fig. 2, it can be seen that no other peaks were eluted besides the two active compounds. The method was therefore considered to be specific.

There are two advantages in this method development over the earlier reported methods. One is solvent system, a lower proportion of ethyl acetate and methanol was used for the method development of Lamivudine and Zidovudine instead of a higher proportion of chloroform and methanol as reported in earlier methods [8,11]. Second is lower limits of detection and

quantification, they were 2.23 ng/spot and 7.90 ng/spot for Lamivudine and 2.98 ng/spot and 8.85 ng/spot for Zidovudine, which are lower than those reported earlier by Habte et al. [8] and Sockalingam et al. [11]. By adjusting the relative proportion of ethyl acetate and methanol reasonable retention time for Lamivudine and Zidovudine was obtained with good peak shape.

### 3.6. *Results of analysis of tablets formulations*

Analysis of samples of marketed antiretroviral tablets containing Lamivudine 150 mg and Zidovudine 300 mg was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recovery of Lamivudine and Zidovudine was $99.26 \pm 1.31$ and $99.14 \pm 1.14$, respectively, and these values are complying with the assay specifications for active drugs in the United States of Pharmacopoeia (90.0–110.0%) [12], which are required to be met by most drug formulations.

## 4. Conclusion

A quick, precise and accurate method based on normal-phase HPTLC has been developed for routine analysis of Lamivudine and Zidovudine in fixed-dose combination tablets. The method was validated for linearity, precision, accuracy and specificity. It has the advantage over HPLC methods in general. It consumed less than 35 mL of mobile phase per run (8 samples per plate), whereas HPLC methods would consume more than 50 mL per runs of similar number of samples. If we consider the time from sample preparation to densitometric evolution for one plate, the new method took an average of 1 h, whereas HPLC methods would generally take more than 2 h for the same number of samples. It is cheap, quick and does not use chloroform, therefore suitable for routine analysis of Lamivudine and Zidovudine in fixed-dose combination tablets. When compared with the reported HPLC method, the developed HPTLC method is both time and cost effective for the determination of Lamivudine and Zidovudine mixtures.

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