

# Desitometric Development and Validation for Simultaneous Determination of Antiretroviral Drugs Lamivudine, Zidovudine and Nevirapine in Fixed-Dose Combination Tablets

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## ABSTRACT

A cutting-edge high-performance thin layer chromatography (HPTLC) approach designed for simultaneous measurement of tablets containing drugs like lamivudine, nevirapine, and zidovudine. Chromatograms were performed employing chloroform and methanol (8:2 v/v) on pre-coated silica gel aluminum thin-layer chromatography (TLC) plates. The data was quantified using the optical density absorbance mode at 271 nm. The R<sub>f</sub> values for lamivudine, zidovudine, and nevirapine were 0.140, 0.523, and 0.678, respectively. Linearity was found for lamivudine, zidovudine and nevirapine at concentrations of 150 to 900 ng band<sup>-1</sup>, 300 to 1800 and 200 to 1200 ng band<sup>-1</sup>, respectively. Lamivudine had a lower limit of detection (LLoD) of 27.36 ng band<sup>-1</sup> and a lower limit of quantitation (LLoQ) of 82.91 ng band<sup>-1</sup>, whereas zidovudine had LoQs of 64.13 ng band<sup>-1</sup> and 194.34 ng band<sup>-1</sup>. For nevirapine, the values were 35.52 and 107.64 ng band<sup>-1</sup>, respectively. Lamivudine had a lower LoD of 27.36 ng band<sup>-1</sup> and a lower LoQ of 82.91 ng band<sup>-1</sup>, whereas zidovudine had LoQs of 64.13 and 194.34 ng band<sup>-1</sup>. For nevirapine, the values were 35.52 and 107.64 ng band<sup>-1</sup>, respectively. The recovery, specificity, and accuracy of this device have been demonstrated. This new formulation evaluated market tablet formulations of the above drugs (Duovir, Cipla Ltd.). The developed method demonstrated the ability to simultaneously identify these drugs from quantitative data without interference.

**Keywords:** HPTLC, Validation, Lamivudine, Nevirapine, and Zidovudine, Anti-viral.

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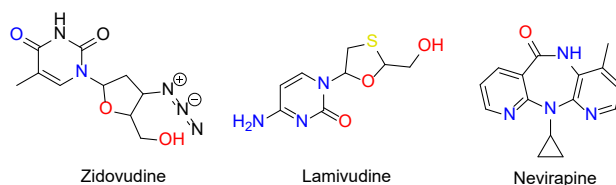
**Conflict of interest:** None

## INTRODUCTION

Human immunodeficiency virus (HIV)/Acquired immunodeficiency syndrome (AIDS) is a severe threat to the globe, with significant social, economic, and moral ramifications.<sup>1,2</sup> It is currently the biggest cause of mortality globally. According to UNAIDS/WHO, approximately 39 million people will be living with HIV by 2022-23.<sup>3,4</sup> However, new antiretroviral drugs are still needed to overcome the increase in resistance and the many dangers associated with long-term antiretroviral use.<sup>5,6</sup> Antiretroviral medications include lamivudine, zidovudine, and nevirapine (Figure 1). It is often used in HIV therapy, particularly in low-income nations.<sup>7,8</sup>

The synthetic binary nucleoside analogs lamivudine and zidovudine were first shown to be effective alone or in combination with other medicines.<sup>9,10</sup> A review of the literature found the presence of analytical techniques for detecting these chemicals in biological products, medicines, and quantitative data. The increased availability of both vaccines has led to the development of safe and effective human immunodeficiency

virus (HIV-1) vaccines and the introduction of new non-nucleoside drugs, the NRTI nevirapine.<sup>11,12</sup> While the prodrugs lamivudine and zidovudine require intracellular activation, nevirapine exerts its effects by directly inhibiting RNA-DNA-dependent DNA polymerase.<sup>13</sup> High-performance thin layer chromatography's (HPTLC) more efficient and sensitive performance has been widely acknowledged. However, many pharmaceutical businesses continue to employ high-performance liquid chromatography (HPLC) for quality analysis. HPTLC offers the benefit of analyzing several samples concurrently with fewer transportable platforms. The



**Figure 1:** Chemical structure of non-nucleoside reverse transcriptase inhibitor

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rationale of the current study was to create and verify a simple, dependable, and accurate HPTLC technique for detecting lamivudine, zidovudine, and nevirapine combinations in preparations.

## MATERIALS AND METHODS

### HPTLC Device

In this study, devices and equipment such as CAMAG (Made in Switzerland) (version 1.4.2) equipped with Linomat 5 applicator (version 1.4.2), 100  $\mu\text{L}$  size Hamilton syringe, TLC Scanner III (Optical) were used density scan), using the Win CATS program and CAMAG double chamber, with dimensions of  $10 \times 10$  cm and  $20 \times 10$  cm. UV detection was performed using an ultrasonic measuring device from ENERTECH Electronics Private Limited (Mumbai, Maharashtra) and a Shimadzu UV-visible dual-beam spectrophotometer (Model - 1780) with a monochromator. The size of the HPTLC aluminum plate was  $10 \times 10$  cm and was pre-coated with 250  $\mu\text{m}$  thick silicon RP-18 F254S purchased from Merck, Germany.

### Chemicals and Reagents

All sample chemicals were purchased from Merck & Co., Germany. Solvents and mobile phases (chloroform, n-hexane, and methanol) were of HPLC analytical grade. Merck Mumbai obtained the ammonia (25%) solution and double distilled water. The name of the preparation available on the market is Duovir-N (batch number - BA2087), purchased from a pharmacy.

### Sample Application

Following the prior treatment, plates were rinsed with methanol and then further subjected to drying at  $110^\circ\text{C}$ . Then, the lamivudine, zidovudine and nevirapine samples in the samples and tablets were run on the previously coated surface. silica F254S plates to form 6 mM bands with 9 mM space between each band. Continuous nitrogen sufficient to dry the strip to facilitate application.

### Optimization of mobile time

Chromatography was done with lamivudine ( $150 \mu\text{g mL}^{-1}$ ), zidovudine ( $300 \mu\text{g mL}^{-1}$ ), and nevirapine ( $200 \mu\text{g mL}^{-1}$ ) working solution as the standard. In order to obtain the required properties, the tests were first carried out on standard TLC plates using different concentrations of different solvents. The acceptability of the solvent mixture is measured by tail, surface quality, sensitivity analysis and development time. Various saturation times of the TLC chamber were tested. Tried and tested linear color development using chloroform: methanol (8:2 v/v) can be satisfactory resolution and peak parameters. The chamber was saturated at  $25 \pm 1^\circ\text{C}$  for 30 minutes and the relative humidity was maintained at  $35 \pm 5\%$ . The color difference in the ADC2 chamber is 90 mM and the recording time is 15 minutes.

### Densitometric Analysis

After scanning with deuterium light at 400 to 200 nm, the densitometric TLC Scanner III was used in absorbance

reflection mode to scan at 271 nm. The sample size was  $6.0 \times 0.45$  mm, and the measurement speed was 20 mm/s. Plot the peak area against the hypothesis to create a regression equation and measure each group's area and peak height.

### Drug preparation method

To produce the medicine at a concentration of  $1000 \mu\text{g mL}^{-1}$ , dissolve 10 mg of drug in solvent (10 mL). Using the same process, a workable solution of lamivudine 150, zidovudine 300 and nevirapine  $200 \mu\text{g mL}^{-1}$  was made using methanol.

### Calibration curve

Transfer the standard solution volume ( $5\text{--}30 \mu\text{L band}^{-1}$ ) on the TLC plate in triplicate, then create a calibration curve from the peak area. The process was carried out in range of 500 to 3000 ng  $\text{band}^{-1}$ .

### Validation

Validation of the analytical technique follows ICH Q2 (R1) requirements.<sup>14-16</sup>

- *Linearity*

From standard solutions of lamivudine, zidovudine and nevirapine ( $1000 \mu\text{g mL}^{-1}$ ), prepare a solution containing 150, lamivudine, 300 zidovudine and  $200 \mu\text{g mL}^{-1}$  solution resp. To produce different lines, nevirapine was applied to TLC plates at different concentrations in 6 replicates of each concentration. Linearity (representing the relationship between peak area and concentration) was observed in the range of 150 to 900, 300 to 1800 and 200 to 1200 ng  $\text{band}^{-1}$  for lamivudine, zidovudine and nevirapine, respectively.

- *Accuracy*

Multiple experiments were conducted to assess the method's accuracy by adding reference medicines to samples at three different concentrations: 50, 100, and 150%. The chosen samples included 300, lamivudine, 600 zidovudine and 400 ng  $\text{band}^{-1}$  of nevirapine respectively in the sample solution. Solutions were applied to the TLC plate three times to produce a density plot, and chemical concentrations were determined by equilibrating each component separately.

- *Precision*

Intraday and interday studies provide evidence of the precision of the method. Intraday sensitivity was 2, 3, 5  $\mu\text{L}$  for lamivudine at  $150 \mu\text{g mL}^{-1}$  (300, 450, 600 ng  $\text{band}^{-1}$ ) and 2, 3 for zidovudine at  $300 \mu\text{g mL}^{-1}$  (600 ng  $\text{band}^{-1}$ ) is 5  $\mu\text{L}$ . %RSD was calculated with three variations of nevirapine at concentrations of  $200 \mu\text{g mL}^{-1}$  (400, 600, 800), 900, 1200 ng  $\text{band}^{-1}$  and volumes of 2, 3, 5  $\mu\text{L}$ .

- *Limit of detection*

LoD is calculated as:

LoD of lamivudine - 27,362 ng  $\text{band}^{-1}$

LoD of zidovudine - 64,135 ng  $\text{band}^{-1}$

LoD of nevirapine - 35,524 ng  $\text{band}^{-1}$

- *Limit of quantitation*

The Limit of quantitation (LoQ) readings are shown as follows:

LoQ for lamivudine -82,916 ng band<sup>-1</sup>  
 LoQ for zidovudine -194,347 ng band<sup>-1</sup>  
 LoQ for nevirapine - 107,648 ng band<sup>-1</sup>

• *Specificity*

The method's specificity is validated by maximum purity analysis, which yields a value greater than 0.997, showing no interference from product deterioration or contaminants.

• *Robustness*

The technique's robustness was tested under a variety of situations, including saturation time, mobile phase ratio, development time from the application, scan time from method development, and the impacts on the region were reported.

*Sample solution preparation (analysis preparation)*

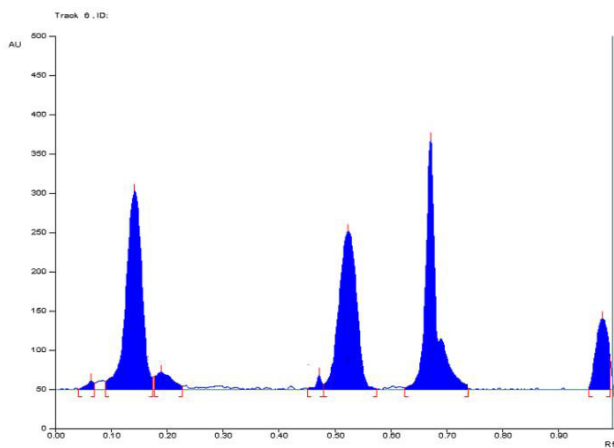
After weighing, ten pills of 200 mg nevirapine, 150 mg lamivudine and 300 mg zidovudine are powdered. The flour was then added to a 10 mL volumetric flask. After that, dilutions were performed using methanol and then sonicated for 10 minutes. Use the same solvent to adjust the volume and filter the solution. Dilutions were performed to achieve final concentrations of 150 µg mL<sup>-1</sup> lamivudine, 300 µg mL<sup>-1</sup> zidovudine, and 200 µg mL<sup>-1</sup> nevirapine.

*Assay (Formulation Analysis)*

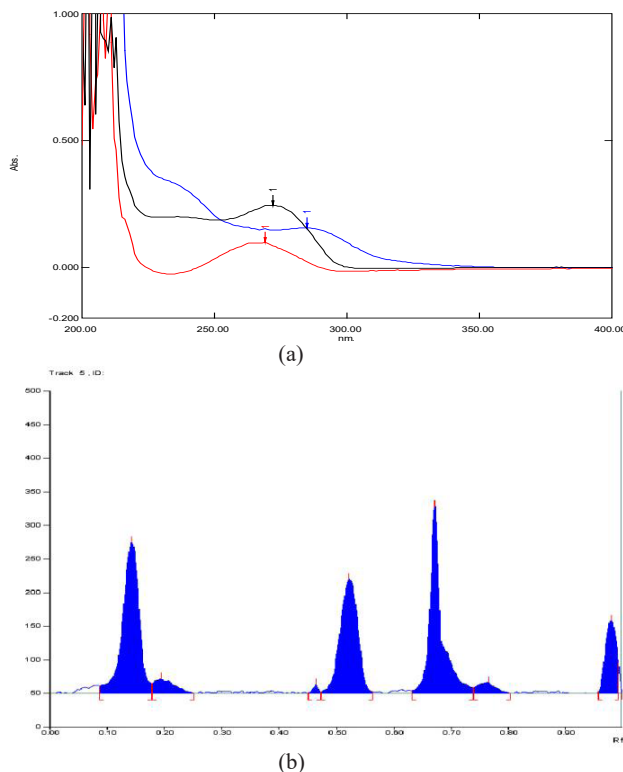
Formulation analysis was performed according to the above procedure and repeated for enough time to fill the field. A linear equation returns the return value. The measured results are shown in a representative chromatogram (ST-1-3) as shown in Figure 2.

**RESULTS & DISCUSSION**

Lami Kev Vudin, zidovudine, and nevirapine using HPTLC developed and validated. All equipment and medications were used as described in the previous section. Densitometric scanning was performed using a Linomat V applicator from CAMAG, a TLC scanner III and a pre-coated aluminum plate (silicon RP-18 F254S). Choose chloroform: methanol (8:2 v/v) solution as the optimized mobile phase. Optical density measurement is used in scanning mode and finds the



**Figure 2:** Intensity of lamivudine, zidovudine and nevirapine (200 ng band<sup>-1</sup>)



**Figure 3:** (a) UV-vis spectra overlaid to display the 271 nm intensity profiles of lamivudine, zidovudine and nevirapine (b) Chloroform solution containing a mixture of lamivudine (300), zidovudine (600), and nevirapine (400) ng band<sup>-1</sup> resp. in a methanol solution (8:2 v/v).

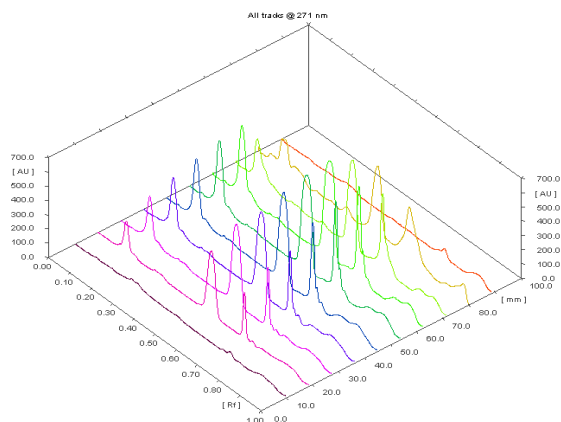
**Table 1:** HPTLC- final chromatography conditions

Chromatographic parameters	HPTLC
Time for development	15 minutes
Temperature	25°C
Wavelength	271 nm
Linearity range	150–900 ng band <sup>-1</sup> (Lamivudine) 280–1800 ng band <sup>-1</sup> (Zidovudine) 200–1300 ng band <sup>-1</sup> (Nevirapine)
R <sub>f</sub>	0.140 ± 0.000 (Lamivudine) 0.523 ± 0.987 (Zidovudine) 0.678 ± 1.723 (Nevirapine)
Diluent	Methanol
Mobile phase	Chloroform: Methanol (8: 2 v/v)

wavelength in the UV 400 ~ 200 nm area. The absorbance at 271 nm is obtained from the overlap of nevirapine, zidovudine and lamivudine (Figure 3a and 3b).

The R<sub>f</sub> values for lamivudine, zidovudine, and nevirapine are 0, 0.14, 0.52, and 0.67, respectively. The chamber is saturated at 25 ± 1°C and 35 ± 5% relative humidity for 30 minutes. Color development distance and time in the ADC2 chamber are 80 mM and 15 minutes, respectively. The final chromatographic data and 3D density are shown in (Table 1) and (Figure 4), respectively.

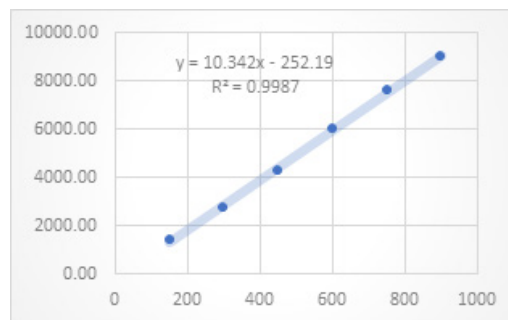
The calibration curve spans a linear range of 150 to 900 for lamivudine, 300 to 1800 for zidovudine, and 200 to 1300



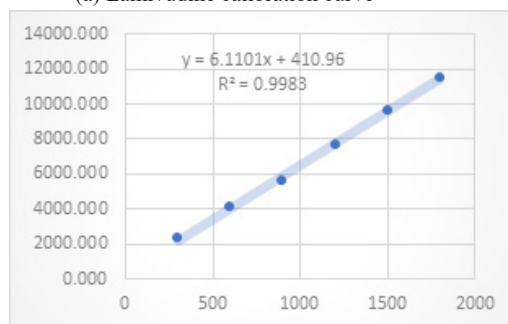
**Figure 4:** Linear overlay 3D density plot of lamivudine, zidovudine and nevirapine

ng band<sup>-1</sup>, respectively for nevirapine. The linear correlation coefficients (*r*<sup>2</sup>) for ng band<sup>-1</sup> are 0.9987, 0.9983, and 0.9976, as illustrated in Figure 5.

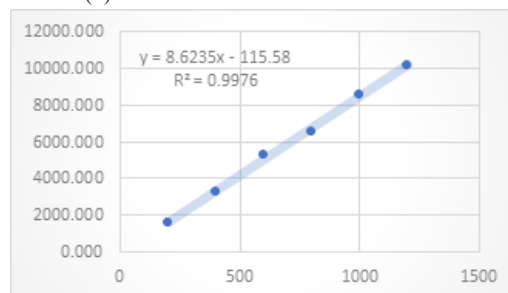
The sensitivity, robustness, and accuracy of this approach have been demonstrated. Repeated tests at various levels were performed to prove the method's accuracy, including 50, 100, and 150%. The recovery rates for lamivudine, nevirapine and zidovudine were reported to be 99.38 to 100.02% w/w. In the daily variation study, the same procedure is applied every day for three consecutive days to calculate %RSD. Daily variable values are shown in Table 2. The detection and quantity limits of lamivudine, zidovudine and nevirapine are 27.36, 64.13 and 35.52 ng band<sup>-1</sup> and 82.91, 194.34 and 107.64, respectively. Strength studies were carried out at different wavelengths ± 1 minute, room saturation point ± 1 minute and the duration from the 1<sup>st</sup> application to the development phase and the 2<sup>nd</sup> application phase. The increase in scan time is 0, 30 and 60 minutes, respectively.



(a) Lamivudine calibration curve



(b) Zidovudine calibration curve



(c) Calibrated nevirapine smooth curve diagram.

**Figure 5:** Displays the calibration curves for lamivudine (a), zidovudine (b), and nevirapine (c) in ng band<sup>-1</sup>, with nevirapine ranging from 200–1200 ng band<sup>-1</sup>.

**Table 2:** Summary of validation parameters

S. No.	Validation parameter	Results		
		Lamivudine	Zidovudine	Nevirapine
1.	Assay (Mean ± RSD)	99.974 ± 0.583	100.137 ± 0.621	100.179 ± 0.234
	Accuracy (%Recovery)	Mean %Recovery ± RSD		
	50	99.387 ± 0.277	99.831 ± 0.171	101.136 ± 0.395
2.	100	99.895 ± 0.688	99.670 ± 0.739	100.545 ± 0.657
	150	100.024 ± 0.091	100.118 ± 0.159	100.282 ± 0.417
3.	Linearity	y = 10.342x - 252.19 R <sup>2</sup> = 0.9987	y = 6.1101x + 410.96 R <sup>2</sup> = 0.9983	y = 8.6236x - 115.58 R <sup>2</sup> = 0.9976
4.	LoD (ng band <sup>-1</sup> )	27.362	64.135	35.524
5.	LoQ (ng band <sup>-1</sup> )	82.916	194.347	107.648
	Precision	%RSD	%RSD	%RSD
6.	Intraday precision	0.543–0.744	0.134–1.010	0.362–0.809
	Interlay precision	0.197–0.706	0.599 – 0.844	0.239–0.608
7.	Range (ng band <sup>-1</sup> )	150–900	300–1800	200–1200
8.	Robustness	Robust	Robust	Robust
9.	Specificity	Within limit	Within limit	Within limit

## DISCUSSION

After a comprehensive review of the available literature, we determined the optimal time for separating and analyzing lamivudine, zidovudine, and nevirapine based on solvent polarity. Many solvents have been investigated in selected fractions to obtain a good solution.

Following the completion of the experiment, the preferred level was determined to be an 8:2 (v/v) blend of chloroform and methanol. This optimized composition aids in the separation of target compounds, with separate bands containing R<sub>f</sub> values of 0.14, 0.52, and 0.67 for lamivudine, zidovudine, and nevirapine. Further investigation of the building approach showed the range of indicators. Lamivudine ranges from 150 to 900 ng band<sup>-1</sup>, zidovudine from 300 to 1800 ng band<sup>-1</sup>, and nevirapine from 200 to 1200 ng band<sup>-1</sup>. Validation accuracy exceeds 99.38%. It may be discovered up to 100.54%, indicating that the procedure is accurate and reliable.

Furthermore, quality assurance procedures were carried out to determine different characteristics such as percentage standard deviation (%RSD), LoD, LoQ and potency. All evidence met predefined criteria, increasing the robustness and validity of the developed screening process.

## CONCLUSION

The verification process confirmed the robustness and efficiency of the design. Its efficiency in providing rapid results indicates its suitability for the evaluation period. In addition, the accuracy and reliability of the method give confidence in its application in various drug formulations.

One of the key features of this method is its sensitivity, which allows the detection and identification of a large number of target compounds. This sensitivity enables the method to reliably detect lamivudine, zidovudine and nevirapine, which are important for research and management.

Furthermore, drug formulation recovery study showed that the approach could identify the proper target despite the presence of other medications, which are frequently contained in formulations. This suggests that excipients impact the approach less, therefore boosting its practical use.

In conclusion, the effective invention and validation of this RP-HPTLC technique represents a substantial addition to the field of analytical chemistry, particularly the analytical sector. Its simplicity, precision, accuracy, reliability, precision, and robustness make it essential for the analysis of lamivudine, zidovudine, and nevirapine in quantitative data and medical preparations, providing the pharmaceutical industry with a dependable approach to regulatory compliance and quality assurance.

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