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Artificial Intelligence in Healthcare: Transforming Diagnosis, Treatment, and Patient Outcomes

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Abstract

Background: A fast, reliable, and sensitive RP-HPLC method was developed for the simultaneous quantification of Lamivudine (3TC), Tenofovir (TFV), and Efavirenz (EFV) in pure form and their combined pharmaceutical formulations.

Methods: The analysis was performed on an Inertsil ODS-3V column (250 × 4.6 mm, 5 µm) using an isocratic mobile phase composed of Acetonitrile and 1% Isopropyl Alcohol (IPA) in the ratio 85:15, flowing at 1 mL/min. Detection was carried out at 256 nm with a PDA detector.

Results: Retention times for 3TC, TFV, and EFV were observed at 2.4, 2.8, and 4.5 minutes, respectively (± 0.5 min). The method showed excellent linearity for all three drugs with correlation coefficients (R^2) of 0.999. Recovery studies ranged between 98% and 102%, and precision (%RSD) values were below 2%. The method's sensitivity was confirmed by determining the limits of detection (LOD) and quantification (LOQ), which were 0.06, 0.09, 0.17 µg/mL and 0.18, 0.27, 0.53 µg/mL for 3TC, TFV, and EFV, respectively.

Conclusion: This RP-HPLC method is rapid, robust, and highly reproducible, making it suitable for routine quality control and high-throughput analysis of these antiretroviral drugs in combined dosage forms. Its simplicity and accuracy ensure reliable application in pharmaceutical laboratories.

KEYWORDS: **Lamivudine; Tenofovir; Efavirenz; RP-HPLC; ICH guidelines; simultaneous estimation.**

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INTRODUCTION

Lamivudine (3TC), chemically known as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one, is a nucleoside analogue and reverse transcriptase inhibitor used in the management of Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) infections [1]. After intracellular phosphorylation, 3TC is converted into its active triphosphate form, which competes with natural nucleotides for incorporation into viral DNA. The absence of a 3'-hydroxyl group prevents the formation of the 5' to 3' phosphodiester bond required for DNA chain elongation, thus terminating viral DNA synthesis [2].

Tenofovir (TFV), chemically described as {[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy} methanephosphonate, is an acyclic nucleotide analogue of deoxyadenosine monophosphate (d-AMP) [3]. Similar to 3TC, TFV lacks a 3'-hydroxyl group, which inhibits the formation of phosphodiester linkages during DNA elongation. It acts as a reverse transcriptase inhibitor, blocking viral DNA synthesis and replication [4].

Efavirenz (EFV), or (4R)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3,1-benzoxazin-2-one, is a non-nucleoside reverse transcriptase inhibitor (NNRTI) [5]. EFV binds directly to HIV-1 reverse transcriptase, disrupting RNA-dependent DNA polymerase activity. In addition, EFV induces cytochrome P450

enzymes, influencing drug metabolism. Its action reduces viral load, preserves immune function, and lowers the risk of progression to AIDS [6].

Several RP-HPLC methods have been reported for the simultaneous estimation of 3TC, TFV, and EFV in pharmaceutical formulations [7]. However, most reported methods utilize buffer-based mobile phases. No previous studies have employed isopropyl alcohol (IPA) in the mobile phase for the simultaneous estimation of these drugs. Therefore, the current study aims to develop a novel, rapid, and validated RP-HPLC method using IPA for the simultaneous estimation of 3TC, TFV, and EFV in pure and combined dosage forms [8].

MATERIALS AND METHODS

Solubility Determination

The solubility of Lamivudine (3TC), Tenofovir (TFV), and Efavirenz (EFV) was tested in various solvents. Based on solubility results (Figure 1), a mixture of acetonitrile (ACN) and 1% isopropyl alcohol (IPA) in a ratio of 85:15 was selected for further studies.

Detector Wavelength Selection

A standard mixture containing 100 µg/mL of 3TC, TFV, and EFV was scanned over the UV range (200–400 nm) using a photodiode-array detector. The maximum absorbance (isobestic point) was observed at 256 nm — this wavelength was chosen for detection [9].

Preparation of Standard Solutions

Accurately weighed amounts of 10 mg each of 3TC, TFV, and EFV were transferred into separate 10 mL volumetric flasks. Approximately 4 mL diluent was added to dissolve the drugs completely, and the volume was brought up to 10 mL to get stock solutions (1000 µg/mL).

Working Standard Solutions

From stock solutions, 0.1 mL was pipetted into a 10 mL volumetric flask, sonicated, and diluted to obtain working standard solutions of 10 µg/mL.

Sample Solution Preparation

Twenty tablets were weighed and powdered. A portion of tablet powder equivalent to 1.7 g (label claim basis) was transferred into a 10 mL volumetric flask, ~4 mL diluent was added, sonicated for 15 min, and diluted to 10 mL. The solution was filtered (Whatman filter) to yield the primary stock; then 1 mL was further diluted to 10 mL for assay.

Chromatographic Conditions

Analysis was performed on a Shimadzu HPLC (SPD-M20A) equipped with a C18 column (4.6 × 250 mm, 5 µm), PDA detector and Rheodyne injector. The mobile phase was ACN:1% IPA (85:15 v/v), pumped at 1.0 mL/min, injection volume 20 µL, column temperature 30 °C, detection at 256 nm [10].

Method Validation

- **Linearity:** Calibration standards in ranges of 4–20 µg/mL (3TC), 10–50 µg/mL (TFV), and 8–40 µg/mL (EFV) were prepared, filtered (0.45 µm), injected, and calibration curves constructed. The acceptance criterion was $R^2 \geq 0.999$ [11].
- **Precision (intra-day):** Six replicates of standard solution (3TC 12 µg/mL, TFV 30 µg/mL, EFV 24 µg/mL) were injected on the same day; %RSD required ≤ 2% [12].
- **Intermediate precision (inter-day):** Six replicate injections of the same standard solutions on a different day; %RSD should remain ≤ 2% [13].

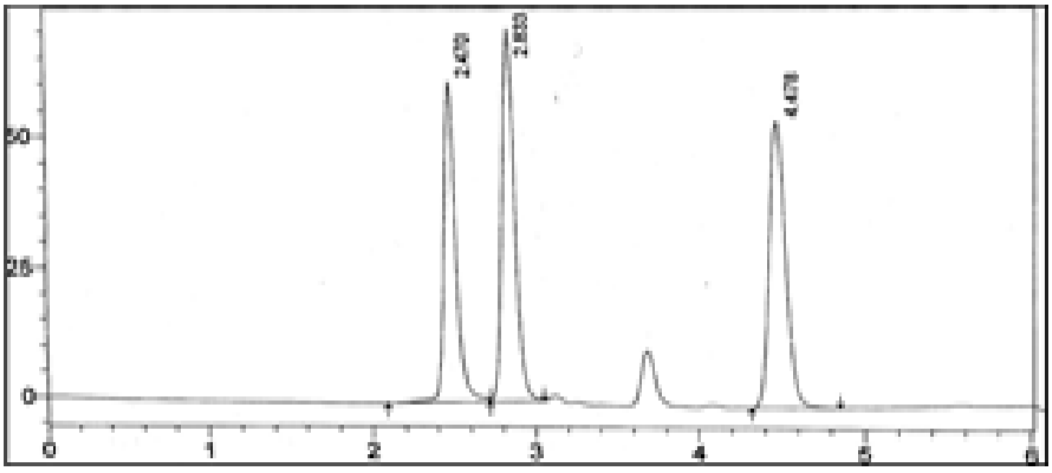

Figure 1: Chromatogram of optimized method.

- **Accuracy (Recovery):** Known amounts of drugs were spiked into sample matrices at 50%, 100%, and 150% levels; triplicate preparations for each level; percentage recovery calculated per ICH guidelines [14].
- **Robustness:** The method's robustness was checked by deliberately varying flow rate (0.8 / 1.2 mL/min), column temperature (25 °C / 35 °C), and detection wavelength (251 / 261 nm), assessing any significant change in retention times or recovery [15] (Table 1).

Table 1: Validation Parameters for Simultaneous Estimation of 3TC, TFV, and EFV by RP-HPLC.

Parameter	3TC	TFV	EFV
Retention Time (min)	2.47 ± 0.05	2.83 ± 0.05	4.48 ± 0.05
Linearity Range (µg/mL)	4 – 20	10 – 50	8 – 40
Correlation Coefficient (R^2)	0.999	0.999	0.999
Limit of Detection (LOD, µg/mL)	0.06	0.09	0.17
Limit of Quantification (LOQ, µg/mL)	0.18	0.27	0.53
Recovery (%)	98 – 102	98 – 102	98 – 102
Precision (%RSD, n=6)	1.2	1.5	1.4
System Suitability (Rs)	>2	>2	>2

Note: *Chromatographic conditions – Column: Inertsil ODS-3V (250 × 4.6 mm, 5 µm); Mobile Phase: ACN:1% IPA (85:15, v/v); Flow Rate: 1 mL/min; Detection Wavelength: 256 nm; Injection Volume: 20 µL.*

Assay of Dosage Form

Tablet powder equivalent to label claim was dissolved, filtered, diluted as described, and injected under optimized chromatographic conditions. Resulting chromatograms were used to quantify 3TC, TFV, and EFV content [10].

RESULTS AND DISCUSSION

Through systematic trials, the HPLC method was optimized for simultaneous quantification of 3TC, TFV, and EFV. Various mobile phase mixtures (ACN:1% IPA at ratios 60:40, 70:30, 80:20, 90:10 v/v) at a flow rate of 1 mL/min were evaluated. Among these, the 85:15 ratio yielded symmetrical peaks and adequate resolution (Rs > 2), with total run time under 5 minutes — rendering it highly efficient for routine analysis. Retention times (RT) for 3TC, TFV, and EFV were observed at 2.47, 2.83, and 4.48 minutes, respectively.

Linearity was demonstrated across 4–20 µg/mL (3TC), 10–50 µg/mL (TFV), and 8–40 µg/mL (EFV), with correlation coefficients (R^2) of 0.999 for each analyte. LOD and LOQ values were estimated from regression parameters: LOD = 0.06, 0.09, 0.17 µg/mL and LOQ = 0.18, 0.27, 0.53 µg/mL for 3TC, TFV, and EFV, respectively [15].

Precision studies produced %RSD values under 2% (intra- and inter-day), confirming method repeatability and reliability. Accuracy via recovery experiments returned values between 98–102%, complying with ICH criteria. Robustness tests (variation in flow rate, temperature, wavelength) did not significantly affect assay results, indicating method stability under typical operational fluctuations.

Compared to previously reported methods, this RP-HPLC approach offers superior speed (short retention times), robust separation, and a simpler mobile phase composition — especially notable due to use of IPA instead of buffer-based systems. This makes it well-suited for routine quality control of combined antiretroviral dosage forms.

In summary, the validated method is simple, accurate, precise, robust, and capable of reliably quantifying 3TC, TFV, and EFV in both bulk drug and finished-dose formulations — making it ideal for routine pharmaceutical analysis and quality assurance workflows.

CONCLUSION

A novel RP-HPLC method for the simultaneous estimation of Lamivudine (3TC), Tenofovir (TFV), and Efavirenz (EFV) was successfully developed and validated using a mobile phase composed of acetonitrile and 1% isopropyl alcohol (IPA). IPA, acting as a co-solvent, enhances selectivity in binary and ternary mixtures and helps maintain column integrity by preventing adsorption of impurities from the sample matrix, which could otherwise alter analyte retention or introduce baseline noise. Its inclusion ensures efficient column washing, minimizes peak broadening, and reduces backpressure issues commonly observed with high organic content. The method demonstrated excellent precision, accuracy, and reproducibility, with low %RSD values, and was capable of detecting and quantifying the analytes across lower concentration ranges than previously reported methods. Overall, this RP-HPLC approach provides a simple, reliable, and robust analytical tool suitable for routine quality control of combined antiretroviral pharmaceutical formulations.

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