Journal of Applied Pharmaceutical Research
Volume 14 Issue 2, Year of Publication 2026, Page 118 – 127
DOI: https://doi.org/10.69857/joapr.v14i2.1505

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

DESIGN AND VALIDATION OF A ROBUST RP-UPLC APPROACH FOR SIMULTANEOUS QUANTIFICATION OF EMTRICITABINE, TENOFOVIR, AND NELFINAVIR IN ACTIVE PHARMACEUTICAL INGREDIENTS AND FINISHED DOSAGE PRODUCTS

Gayathri N[1]*, Kannappan N[2], Srinivasan N[2], Surendra Kumar M[3]

Article Information
Received: 29th November 2025
Revised: 20th January 2026
Accepted: 3rd February 2026
Published: 15th March 2026

Keywords
RP-UPLC, Antiretroviral Drugs, Emtricitabine, Tenofovir, Nelfinavir, Therapeutic Drug Monitoring.

ABSTRACT

Background: Fixed-dose combinations of antiretroviral agents such as Emtricitabine (EMT), Tenofovir (TEN), and Nelfinavir (NEL) are central to Human Immunodeficiency Virus (HIV) therapy. Reliable quantification of these drugs is essential for pharmaceutical quality assurance and therapeutic drug monitoring (TDM) in clinical research. **Methodology:** A reverse-phase ultra-performance liquid chromatography (RP-UPLC) method was developed to simultaneously estimate EMT, TEN, and NEL in bulk and combined dosage forms. Separation was performed on a Waters CHS C18 column using potassium dihydrogen phosphate buffer and acetonitrile (65:35 v/v, pH 3.0) at a flow rate of 0.3 mL/min, with UV detection at 260 nm. Method validation was conducted in accordance with ICH guidelines for linearity, accuracy, precision, sensitivity, robustness, and stability. Forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, and photolytic conditions. **Results and Discussion:** Retention times were 1.004 min (EMT), 1.310 min (TEN), and 1.870 min (NEL), with excellent peak resolution. Recovery studies demonstrated high accuracy, 99.74% (EMT), 99.47% (TEN), and 100.33% (NEL). The method showed strong linearity ($r^2 > 0.999$), low LOD/LOQ values, and robustness. Stability-indicating potential was confirmed via forced degradation studies. The technique proved to be rapid, precise, and stability-indicating, making it suitable for routine pharmaceutical quality control. Its sensitivity and robustness suggest potential utility in biomedical applications, including TDM and pharmacokinetic studies in HIV patients. **Conclusion:** The validated RP-UPLC method enables accurate, precise, and rapid estimation of EMT, TEN, and NEL, supporting its application in both pharmaceutical and clinical research settings.

[1]*Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram – 608002, Tamil Nadu, India*
[2]*Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Chidambaram – 608002, Tamil Nadu, India*
[3]*Senghundhar College of Pharmacy, Namakkal – 637205, Tamil Nadu, India.*

For Correspondence:** **gayathriphd12@gmail.com

INTRODUCTION

The global impact of Human Immunodeficiency Virus (HIV) has necessitated the widespread implementation of combination antiretroviral therapy (cART), which employs agents from multiple pharmacological classes to achieve sustained viral suppression and delay disease progression. Among these, Emtricitabine, Tenofovir, and Nelfinavir are frequently utilized due to their complementary mechanisms of action and proven synergistic efficacy. Ensuring accurate quantification of these drugs in combined dosage forms is critical for maintaining quality control, dose uniformity, and therapeutic reliability [1]. Emtricitabine is a synthetic nucleoside analogue that functions as a reverse transcriptase inhibitor, disrupting viral replication by incorporating into viral DNA and causing chain termination. It has a molecular weight of 247.24 g/mol and a chemical formula of $C_8H_{10}FN_3O_3S$. This compound is notably water-soluble, with a solubility of approximately 112 mg/mL at room temperature [2]. Tenofovir is a nucleotide reverse-transcriptase inhibitor that structurally resembles adenosine monophosphate, lacking the cyclic sugar ring, thereby enhancing its ability to inhibit reverse transcription. Its primary mechanism involves blocking the reverse transcription process to suppress HIV replication. The parent compound has a molecular formula of $C_9H_{14}N_5O_4P$ and a molecular weight of 287.21 g/mol. Tenofovir demonstrates an aqueous solubility of approximately 4.86 mg/mL and moderate lipophilicity, with a log P value of 1.6. To overcome the poor oral bioavailability of tenofovir, prodrug forms such as tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) are used clinically.

TDF ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$; ~635 g/mol) and TAF ($C_{21}H_{29}N_6O_5P$; 476.47 g/mol) improve pharmacokinetic properties while releasing tenofovir in vivo. In the present work, analytical method development and validation were conducted for the parent compound, tenofovir, rather than its prodrug derivatives. [3]. Nelfinavir, a protease inhibitor, plays a crucial role in antiretroviral therapy by targeting the HIV-1 and HIV-2 proteases, which are essential for viral particle maturation. It is a chemically complex molecule with the formula $C_{32}H_{45}N_3O_4S$ and a molecular weight of 567.78 g/mol. Nelfinavir is characterized by low water solubility and high lipophilicity, with two ionizable groups displaying pKa values of 9.32 and 8.18, properties that significantly influence its formulation and absorption profiles [4]. While analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) are available for estimating individual anti- retrovirals, there is currently no validated reverse-phase UPLC method capable of simultaneously quantifying Emtricitabine, Tenofovir, and Nelfinavir. Given the increasing use of these agents in fixed-dose combination therapies, developing a unified analytical method would greatly enhance quality assurance by reducing analysis time, solvent use, and operational costs [5].

Analytical methods for the determination of Emtricitabine (EMT), Tenofovir (TEN), and Nelfinavir (NEL), either individually or in various combinations, have been reported in the literature using techniques such as RP-HPLC, RP-UPLC, LC/MS/MS, and UPLC–MS/MS. However, no studies to date have described a reverse-phase UPLC method for the simultaneous estimation of EMT, TEN, and NEL. Considering that these three agents are commonly co-formulated for the treatment of HIV, there is a clear need for such an analytical approach. To address this gap, a robust reverse-phase UPLC method was systematically developed and validated in accordance with ICH guidelines, facilitating the concurrent quantification of EMT, TEN, and NEL in pharmaceutical dosage forms [6].

MATERIAL AND METHODS

Chemicals and reagents

M/s. Mylan Laboratories Pvt. Ltd., Hyderabad, India, generously supplied the standard reference materials for emtricitabine, tenofovir, and nelfinavir. M/s. Rankem Chemicals Ltd., in Mumbai, India, provided analytical-grade dihydrogen orthophosphate, orthophosphoric acid, and UPLC-grade acetonitrile. During the study, Milli-Q water was used, and a 0.22 µm filter was employed. Tablets labeled with claims of 200 mg EMT, 300 mg TEN, and 625 mg NEL were prepared in our laboratory for testing.

Instrumentation

Chromatographic analysis was performed using a WATERS UPLC system equipped with an automated injector and a TUV detector, all operated via Empower 2 software. Separation of analytes was accomplished on a Waters CHS C18 analytical column. Spectral evaluations were conducted using a LAB INDIA double-beam UV-visible spectrophotometer. For accurate weighing, a Denver electronic balance was used, and pH adjustments and measurements were performed with a calibrated pH meter from BVK Enterprises India. This

comprehensive setup ensured precise & reproducible analytical performance throughout the study, maintaining stringent control over all instrumental parameters to support method validation [7].

Chromatographic Conditions

Before analysis, solutions were degassed by sonication for 5 minutes and then filtered through a 0.45 µm membrane filter to remove particulates. Chromatographic separation was performed at a constant flow rate of 0.3 mL/min, with detection at 260 nm. The column temperature was carefully maintained at 30 °C to ensure optimal resolution and reproducibility. These parameters were selected to achieve sharp, well-defined peaks and minimize variability across runs. The controlled conditions significantly enhanced the robustness and precision of the developed RP-UPLC method, supporting its application for the simultaneous estimation of the studied antiretrovirals [8].

Phosphate Buffer Preparation

The phosphate buffer was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in approximately 900 mL of Milli-Q water in a 1-liter volumetric flask. The solution was sonicated to remove dissolved gases. Subsequently, 1 mL of triethylamine was added, and the pH was adjusted to 3.0 with a dilute orthophosphoric acid solution. Finally, the volume was brought up to the mark with Milli-Q water. This buffer provided a stable environment, essential for achieving consistent chromatographic separation and minimizing fluctuations caused by pH changes during analytical runs [9].

Mobile Phase Composition

The mobile phase for the chromatographic analysis was prepared by mixing phosphate buffer and acetonitrile at a 65:35 (v/v) ratio. This specific composition was optimized to facilitate effective elution and clear separation of Emtricitabine, Tenofovir, and Nelfinavir. Maintaining this ratio was critical for achieving distinct, sharp peaks with minimal tailing and for ensuring reproducibility across analytical batches. The prepared mobile phase was thoroughly mixed and degassed before use, thereby enhancing the stability and performance of the RP-UPLC system during simultaneous estimation.

Preparing the Diluent

Calibration solutions at target concentrations of 200 µg/mL, 30 µg/mL, and 625 µg/mL were prepared by accurately pipetting 1 mL of the respective stock solutions into separate 10 mL volumetric flasks. Each solution was sonicated to ensure complete dissolution, then diluted to volume with the designated diluent. This procedure ensured homogeneity and consistency across calibration standards, which is crucial for generating reliable calibration curves. The prepared solutions were stored appropriately and used within validated stability timeframes to maintain analytical integrity throughout the quantification process [10].

Making Stock Solutions

Precisely weighed amounts of 20 mg of Emtricitabine, 30 mg of Tenofovir, and 62.5 mg of Nelfinavir were transferred into a clean, dry 50 mL volumetric flask. After thorough mixing to achieve uniform dispersion, the solution was brought to volume using the selected diluent. From this stock, standard solutions at concentrations of 200 µg/mL, 300 µg/mL, and 625 µg/mL were prepared by transferring 1 mL of the stock into 10 mL volumetric flasks and diluting to volume. These carefully prepared solutions served as references for method validation, ensuring accurate and reproducible quantification of the antiretroviral agents across all analytical runs [11].

Setting up a sample solution

A batch of twenty commercial tablets was collected and ground into a fine powder. From this, a quantity equivalent to the weight of one tablet was accurately measured and transferred into a 100 mL volumetric flask containing 70 mL of diluent. The mixture was sonicated for approximately 10 minutes to ensure full dissolution of the active pharmaceutical ingredients. Afterward, the solution was diluted to the 100 mL mark with the same diluent. The final mixture was passed through a 0.45 µm membrane filter to obtain a clear filtrate, which was then used for subsequent analysis. To identify the most suitable wavelength for detection, a UV scan was performed over 200-400 nm. The wavelength of 260 nm was chosen because it yielded the highest absorbance for all three drugs [12].

Preparation of Sample stock solutions of EMT and TEN

The average tablet weight was calculated by weighing 10 tablets individually. A quantity of powder equivalent to the weight of a single tablet was accurately measured and transferred into a 100 mL volumetric flask. Subsequently, 25 mL of diluent was added, and the mixture was sonicated for 10 minutes to ensure complete dissolution of the active pharmaceutical ingredients.

After sonication, the solution was brought to volume with additional diluent. The resulting mixture was then filtered to obtain a clear solution. This final sample preparation yielded concentrations of 2000 µg/mL for emtricitabine and 3000 µg/mL for tenofovir. This solution, containing 2000 µg/mL of emtricitabine and 3000 µg/mL of tenofovir, is hereafter referred to as Solution A

Preparation of Sample Stock Solution of Nelfinavir

The preparation of the sample stock solution commenced with the individual weighing of ten tablets to determine their average mass, ensuring consistency and reliability in subsequent analyses. A portion of powder equivalent to the weight of one tablet was then accurately transferred into a clean, dry 100 mL volumetric flask. Next, 25 mL of diluent was added, and the mixture was sonicated for 20 minutes to ensure complete dissolution of the active pharmaceutical ingredient. This step facilitated uniform dispersion and eliminated undissolved particles. After achieving full dissolution, the solution was diluted to volume with the same diluent and subsequently filtered through a membrane filter to remove any residual particulates. The resulting clear solution, containing 6250 µg/mL of nelfinavir, was appropriately stored for subsequent dilutions and analytical analyses. This solution, containing 6250 µg/mL of nelfinavir, is hereafter referred to as Solution B.

Preparation of Working Standard Solutions

The working standard solutions were prepared by pipetting 0.2 mL each of Solution A (combined EMT and TEN stock solution) and Solution B (NEL stock solution) into a 10 mL volumetric flask using calibrated pipettes to ensure precise volume transfer. The solution was then diluted to volume with an appropriate diluent, resulting in a final concentration of 40 µg/mL for emtricitabine, 60 µg/mL for tenofovir & 125 µg/mL for nelfinavir. These concentrations were selected to fall within the method's validated linearity range and were subsequently used for chromatographic analysis, calibration, and validation.

Method Development

The analytical method was meticulously developed by systematically adjusting critical parameters, including flow rate, mobile-phase composition, and chromatographic column selection, to achieve optimal separation. The final conditions employed a Waters CHS C18 column, using a mobile phase of 0.01 N potassium dihydrogen phosphate (KH_2PO_4) buffer and acetonitrile (65:35). This combination was selected after evaluating various solvent systems to ensure sharp, well-resolved peaks with minimal tailing. The established conditions provided reliable retention times & excellent peak symmetry, thereby supporting the simultaneous estimation of emtricitabine, tenofovir, and nelfinavir in combined dosage forms.

Method Validation

The validated RP-UPLC method facilitated the simultaneous quantification of EMT, TEN, and NEL with high precision and reproducibility. Validation was performed in accordance with ICH guidelines, rigorously assessing parameters such as linearity, precision, specificity, accuracy, robustness, and sensitivity to confirm method suitability. Sensitivity was further demonstrated by determining the limits of detection (LOD) and quantification (LOQ), which established the method's capability to detect even trace levels of analytes. These comprehensive validation results confirmed the method's reliability for routine quality control, ensuring accurate measurement of the targeted antiretrovirals within complex pharmaceutical matrices.

Degradation Studies

The stability-indicating properties of the RP-UPLC method were assessed through forced degradation experiments. The test samples were exposed to a range of stress conditions in accordance with the ICH Q1A(R2) regulatory framework. These circumstances included thermal degradation, oxidative hydrolysis, base hydrolysis, and acid hydrolysis. In accordance with ICH guidelines for assessing light sensitivity, photolytic degradation was evaluated by exposing the sample to ultraviolet (UV) light at 254 nm for 24 hours. To evaluate chemical stability under neutral conditions, the sample was heated with water at 60°C for 2 hours. RP-UPLC was then used to analyze samples subjected to each degradation condition to identify degradation by-products. The peak purity of emtricitabine, tenofovir, and nelfinavir was subsequently evaluated to verify the method's capacity to distinguish the active components from their respective degradation compounds.

RESULT AND DISCUSSION

Results

The RP-UPLC method successfully separated EMT, TEN, and NEL from the mixed standard solution, as shown in Figure 1.

Method validation

The developed RP-UPLC method enabled simultaneous quantification of EMT, TEN, and NEL. It was comprehensively

validated by assessing key analytical attributes, including linearity, specificity, system suitability, accuracy, precision, robustness, and sensitivity limits (LOD and LOQ).

Accuracy

Three levels of recovery experiments using the conventional addition approach were conducted to confirm the method's accuracy. Based on the results shown in Table 1, the mean percentage recoveries for EMT, TEN, and NEL are 99.74%, 99.47%, and 100.33%, respectively. Only the in-house formulation was prepared with common placebo ingredients, and the assay of the three drugs was performed with the validated procedure.

Linearity

The linearity study demonstrated that EMT showed a linear response over the concentration ranges of 10–60 µg/mL, TEN 15–90 µg/mL, and NEL 31.25–187.5 µg/mL. Additional data supporting these findings are presented in Table 2 and Figures 2, 3, and 4.

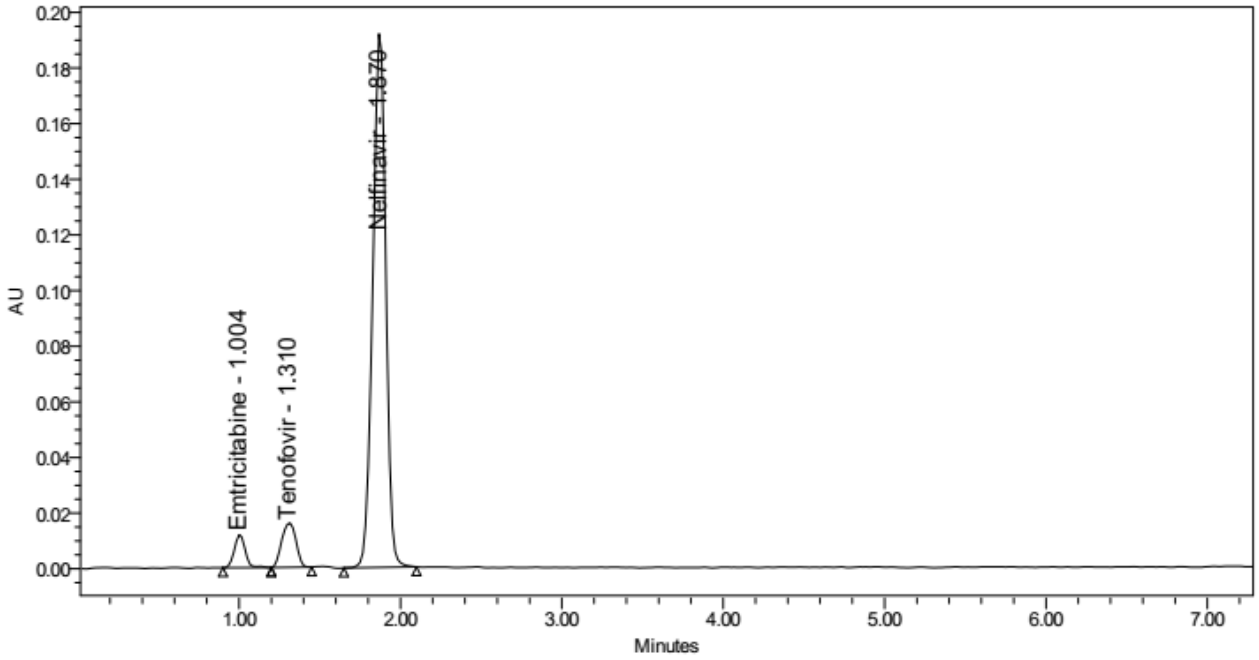

Figure 1: Representative chromatogram illustrating the successful simultaneous separation of emtricitabine, tenofovir, and nelfinavir in the optimized RP-UPLC method.

Table 1: Recovery data of EMT, TEN, and NEL

Pre-analysed amount (µg/mL)			**Spiked amount (µg/mL)**			**% recovered**		
EMT	TEN	NEL	EMT	TEN	NEL	EMT	TEN	NEL
200	300	625	100	150	312.5	99.15	99.18	100.95
200	300	625	100	150	312.5	99.89	100.05	101.35
200	300	625	100	150	312.5	99.27	99.49	100.87
200	300	625	200	300	625	100.56	100.05	101.09
200	300	625	200	300	625	100.36	99.38	99.68
200	300	625	200	300	625	99.90	99.47	99.24
200	300	625	300	450	937.5	99.06	99.46	100.05
200	300	625	300	450	937.5	99.25	99.03	100.54
200	300	625	300	450	937.5	99.63	99.08	99.19
				Mean		99.74	99.47	100.33
				SD		0.66	0.37	0.82
				% RSD		**0.66**	**0.37**	**0.82**

Table 2: Linearity data of EMT, TEN, and NEL

S. No.	EMT		TEN		NEL	
	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1	10	38413	15	70534	31.25	136080
2	20	76540	30	140464	62.5	276875
3	30	114843	45	210458	93.75	419135
4	40	151881	60	281309	125	553856
5	50	189992	75	352837	156.25	683280
6	60	222962	90	418097	187.5	819529

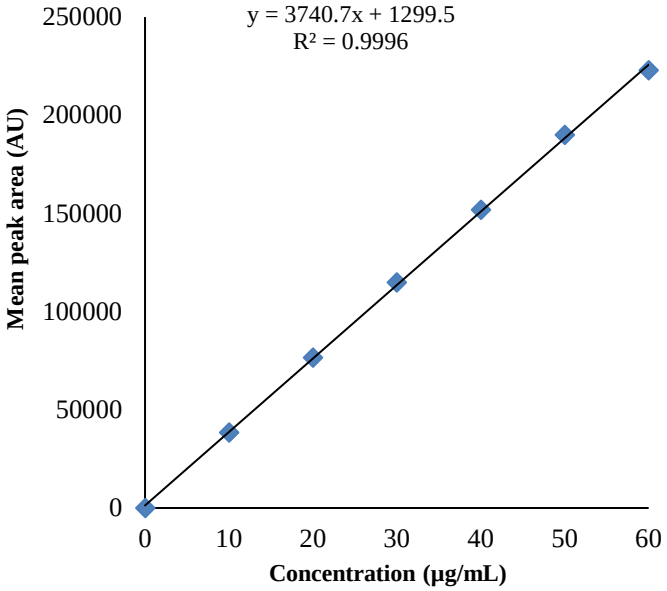

Figure 2: Standard calibration plot demonstrating linear response of emtricitabine peak area across the tested concentration range.

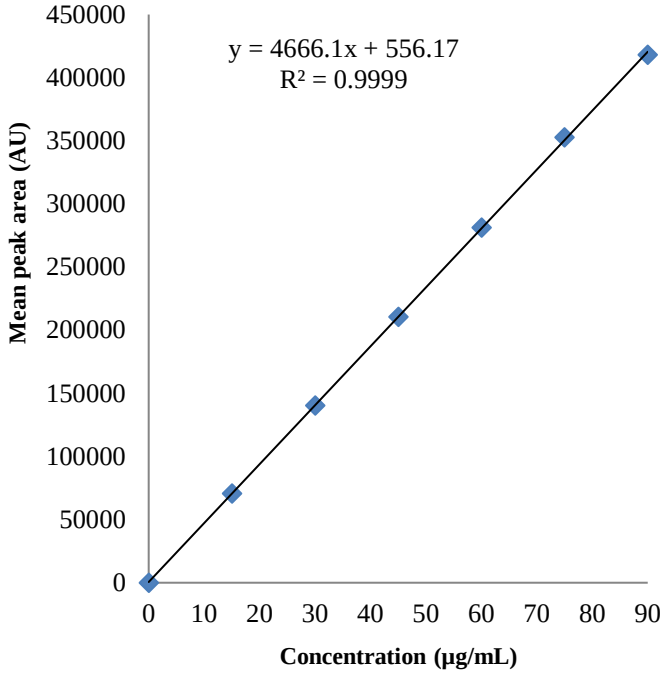

Figure 3: Linear regression plot of tenofovir demonstrating uniform response across the validated concentration range

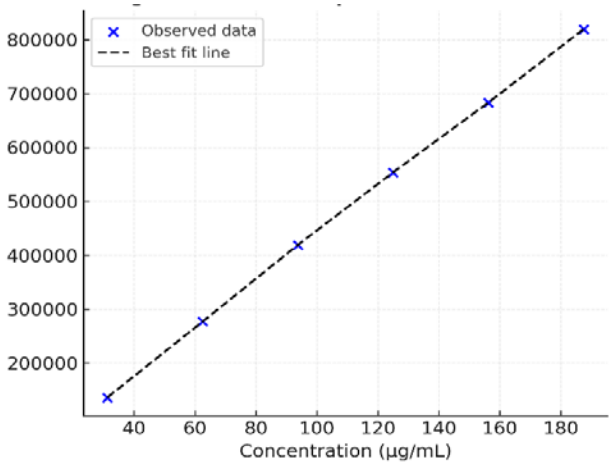

Figure 4: Standard calibration plot of Nelfinavir (NEL)

Precision

The repeatability of an analytical process under standard operating conditions is known as precision. To assess system precision, a measure of procedure variability that can be predicted for a given analyst, six replicate analyses of the same working dilution were carried out. Relative standard deviation percentages for EMT, TEN & NEL were 0.3, 0.8, 0.4 & respectively, given in Table 3.

Table 3: Precision data of EMT, TEN, and NEL

S.No.	Emtricitabine	Tenofovir	Nelfinavir
1	152357	283798	551675
2	151947	282194	547418
3	152466	282285	548097
4	151697	279215	548992
5	152636	284518	552774
6	152737	279182	550561
Mean	152307	281865	549920
Std. Dev.	405.6	2248.6	2101.5
% RSD	0.3	0.8	0.4

Intermediate precision

Intermediate precision was determined by injecting the same working solution six times over two different days. The resulting

% RSD values, 0.7% for EMT, 0.7% for TEN, and 0.6% for NEL, remained within the acceptable threshold of ≤2%, confirming the method's reliability over time. These results confirm the method's reliability and reproducibility. The detailed data are presented in Table 4.

Table 4: Intermediate Precision data of EMT, TEN, and NEL

S.No.	Emtricitabine	Tenofovir	Nelfinavir
1	151320	278321	553782
2	151476	275559	550459
3	151027	275302	548222
4	152547	278378	550035
5	149295	273825	546742
6	150312	276921	543519
Mean	150996	276384	548793
Std. Dev.	1104.8	1812.2	3508.7
% RSD	0.7	0.7	0.6

Limit of Detection and Quantification

The LOD signifies the minimum detectable analyte concentration under defined conditions, although it may not be quantified precisely [13]. In contrast, the limit of quantification (LOQ) represents the lowest concentration that can be measured with acceptable precision and accuracy under validated conditions. LOD and LOQ were calculated using the standard formulae: LOD = 3.3 × (SD/S) and LOQ = 10 × (SD/S), where SD denotes the standard deviation of the intercept, and S is the slope of the calibration curve. For emtricitabine (EMT), the LOD and LOQ were calculated as 0.14 µg/mL and 0.41 µg/mL, respectively. Tenofovir (TEN) showed corresponding values of 0.22 µg/mL for LOD and 0.67 µg/mL for LOQ. Among the three drugs, nelfinavir (NEL) demonstrated the greatest sensitivity, with an LOD of 0.62 µg/mL and an LOQ of 1.88 µg/mL

Robustness

Robustness evaluates the method's resilience against minor, intentional modifications in experimental settings, ensuring reliability under routine analytical variations. After each modification, a mixed standard solution was injected, and the mean area and % assay values were recorded to assess robustness [14]. These results were used to determine the method's consistency and reliability under varied conditions.

Table 5: Robustness data for EMT, TEN, and NEL

Condition	EMT		TEN		NEL	
	Mean area	**% Assay**	**Mean area**	**% Assay**	**Mean area**	**% Assay**
Optimized	152307	99.36	281865	99.01	549920	99.91
Flow rate: (-) 0.2 mL/min	151913	99.10	285322	100.22	549700	99.87
(+) 0.4 mL/min	153099	99.87	286695	100.70	544983	99.01
Mobile phase: (-) Buffer-acetonitrile (70:30)	154022	100.47	282730	99.31	545071	99.02
(+) Buffer-acetonitrile (60:40)	154579	100.82	289990	101.86	558204	101.38
Column Temperature: (-) at 25°C	152399	99.42	282851	99.35	551130	100.12
(+) at 35°C	155906	101.70	283785	99.68	552363	100.353

Degradation Studies

The capability of the developed RP-UPLC method to effectively separate Emtricitabine (EMT), Tenofovir (TEN), and Nelfinavir (NEL) from their respective degradation products was assessed by subjecting the samples to diverse stress conditions. These included acidic and alkaline hydrolysis, oxidative degradation, thermal stress, and photolytic exposure.

Each stressed sample was prepared and analyzed under established chromatographic conditions to monitor for additional peaks indicative of degradation. The method successfully resolved the parent compounds from their degradation products without interference, thereby demonstrating its stability-indicating nature. This confirms the method's suitability for routine quality control and stability studies of these antiretroviral agents [15]. Degradation sample chromatograms in acid, base, and oxidation conditions are represented in Figures 5-7.

DISCUSSION

The developed RP-UPLC method demonstrated excellent precision, accuracy, and robustness for the simultaneous quantification of Emtricitabine (EMT), Tenofovir (TEN), and Nelfinavir (NEL) in fixed-dose pharmaceutical formulations. The short retention times (< 2 minutes) achieved in this study significantly reduce analysis duration compared to conventional HPLC methods, which typically require 6–12 minutes for similar separations [16]. This improvement enhances throughput

in quality-control laboratories and aligns with the industry's shift toward high-efficiency analytical workflows. The mean percentage recoveries for all three analytes (99.47–100.33%) fell within the acceptance criteria outlined in ICH Q2(R1), confirming the method's accuracy for routine analysis [17].

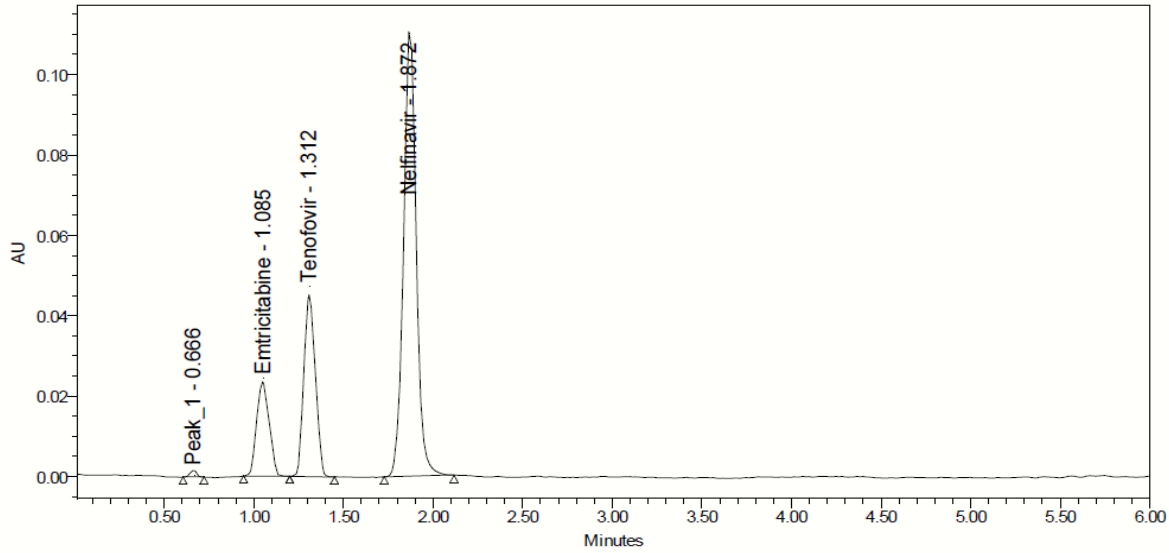

Figure 5: Acid degradation Chromatogram

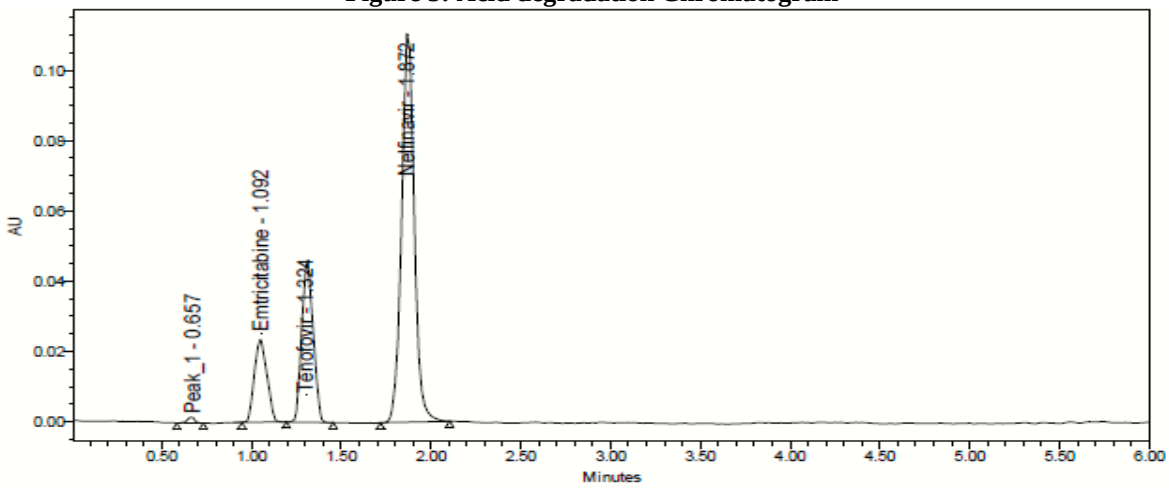

Figure 6: Base degradation Chromatogram

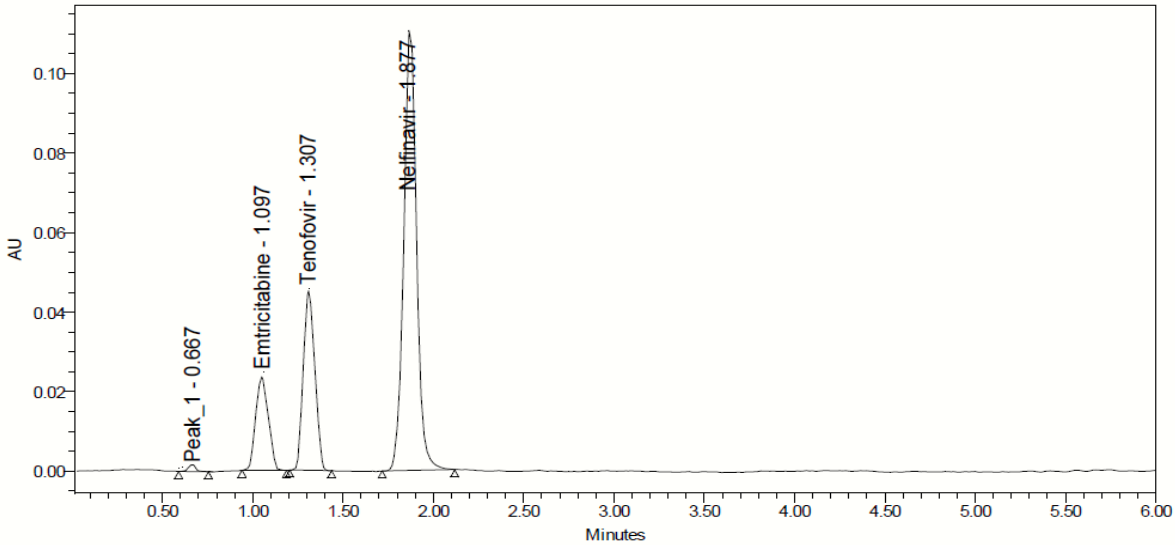

Figure 7: Peroxide degradation Chromatogram

The low %RSD values (<1%) in both system and method precision studies indicate minimal variability, which is consistent with findings from previous chromatographic validation studies of antiretrovirals [18]. LOD and LOQ values indicated high sensitivity, enabling the detection of trace quantities of each drug. This is particularly important for stability studies, where degradation products may be present at very low levels. Forced degradation experiments confirmed the stability-indicating nature of the method, as the active compounds were well resolved from their degradation products under acidic, alkaline, oxidative, thermal, and photolytic stress conditions. Similar approaches have been reported for other protease inhibitors and nucleoside analogues, underscoring the need for stability-indicating assays to ensure regulatory compliance [19].

The mobile-phase composition (phosphate buffer–acetonitrile, 65:35 v/v) and pH were optimized to balance resolution, peak shape, and retention time. Such optimization strategies have been widely recommended for multi-component RP-UPLC methods [20]. The laboratory-prepared dosage forms also yielded assay results for all three drugs within the 98-102% range, indicating the method's applicability. Overall, this method offers a rapid, cost-effective, and regulatory-compliant solution for simultaneously estimating EMT, TEN, and NEL in routine quality control and stability testing. The calibration plots for EMT, TEN, and NEL (Figures 2–4) confirmed excellent linearity across the tested concentration ranges, with correlation coefficients (r^2) greater than 0.999 for all analytes.

CONCLUSION

A robust RP-UPLC method was successfully developed and validated in accordance with ICH recommendations for the simultaneous quantification of emtricitabine, tenofovir, and nelfinavir in fixed-dose combinations. The method demonstrated key strengths, including rapid analysis (retention times < 2 minutes), excellent recovery (close to 100%), strong linearity (r^2 > 0.999), and validated stability-indicating capability under diverse stress conditions. These findings confirm that the method is accurate, precise, sensitive, and reliable for routine quality control and stability testing of combined antiretroviral formulations. One limitation of this work is that validation was performed primarily on laboratory-prepared dosage forms and has not yet been extended to large-scale industrial products or real patient samples. Future studies should focus on applying this method to diverse pharmaceutical formulations and exploring its application in therapeutic drug monitoring. In today's context, where rapid and reliable analytical methods are critical for ensuring the safety and efficacy of life-saving antiretroviral therapies, this validated RP-UPLC method provides a cost-effective, time-efficient, and regulatory-compliant tool. It has the potential to enhance global HIV treatment programs by supporting rigorous quality assurance in both manufacturing and clinical research.

ACKNOWLEDGEMENTS

Special thanks are extended to M/s. Mylan Laboratories Pvt. Ltd., Hyderabad, India, for generously supplying the standard reference materials of Emtricitabine, Tenofovir, and Nelfinavir.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

N. Gayathri conducted the experiments, performed data analysis, and prepared the initial draft of the manuscript. N. Kannappan guided the overall study design and supervised the method development. N. Srinivasan contributed to method validation and assisted with manuscript revision. M. Surendra Kumar provided project administration and gave final approval of the manuscript. All authors reviewed and approved the final version of the work.

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