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Research Article

GREEN REVERSE-PHASE – HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY APPROACH FOR STABILITY-INDICATING SIMULTANEOUS QUANTIFICATION OF DORAVIRINE, LAMIVUDINE, AND TENOFOVIR IN BULK AND DOSAGE FORMS

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ABSTRACT

Objective: A simple, accurate, and validated reverse-phase high-performance liquid chromatography (HPLC) method was developed for the simultaneous estimation of doravirine, lamivudine, and tenofovir in pharmaceutical formulations.

Methods: Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 column (2.1×150 mm, 5 µm) with a mobile phase composed of 0.01 M potassium phosphate buffer (pH adjusted with orthophosphoric acid) and HPLC-grade acetonitrile, delivered at a flow rate of 1 mL/min. Detection was performed at 244 nm.

Results: The method yielded sharp, well-resolved peaks with retention times of 2.195 min for doravirine, 2.715 min for lamivudine, and 3.676 min for tenofovir. A peak resolution of 4.4 confirmed efficient separation. The method exhibited excellent linearity with a correlation coefficient (R^2) of 0.9999 across concentration ranges of 2.515 µg/mL for doravirine and 7.545 µg/mL for both lamivudine and tenofovir. Accuracy was confirmed through recovery studies, yielding 100.02% for doravirine, 100.09% for lamivudine, and 99.98% for tenofovir. The method also demonstrated high sensitivity, with limits of detection of 0.01 µg/mL for doravirine and 0.11 µg/mL for both lamivudine and tenofovir. The respective limits of quantification were 0.04 µg/mL, 0.32 µg/mL, and 0.34 µg/mL. The regression equations derived were as follows: y=30936x +3374.3 for doravirine, y=29559x+889.66 for lamivudine, and y=29768x+3035.2 for tenofovir.

Conclusion: In addition to its strong analytical performance, the method was evaluated using the AGREE metric for green analytical chemistry compliance. Despite the use of acetonitrile, it achieved an AGREE score of 0.73, indicating good environmental compatibility due to minimal solvent consumption, energy efficiency, and avoidance of derivatization. This method – with its short runtime, high accuracy, cost-effectiveness, and environmentally responsible design – is highly suitable for routine quality control and stability studies of antiretroviral pharmaceutical formulations.

Keywords: Doravirine, Lamivudine, Tenofovir, International council for harmonization guidelines, Retention time, AGREE.

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INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome, a chronic and life-threatening condition that progressively weakens the immune system. This immunodeficiency renders individuals highly susceptible to opportunistic infections and various other illnesses. HIV-1 primarily targets helper T cells, especially CD4+ T lymphocytes, along with other critical immune cells such as macrophages and dendritic cells. To manage and suppress HIV-1 infection, combination oral antiretroviral therapy has been developed using a regimen that includes doravirine, lamivudine, and tenofovir. Doravirine, a non-nucleoside reverse transcriptase inhibitor, is chemically described as 3-chloro-5-({1-[(4-methyl-5-oxo-dihydro-1H-1,2,4-triazol-3-yl)methyl]-2-oxo4(trifluoromethyl) -1,2-dihydropyridin-3-yl}), and it plays a key role in inhibiting the replication of the virus by targeting the reverse transcriptase enzyme as shown in Fig. 1 [1-4].

Lamivudine, chemically named (2R, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one, is a synthetic cytidine analog that inhibits the reverse transcriptase enzyme of both HIV-1, HIV-2, and hepatitis B virus. After cellular uptake, lamivudine is phosphorylated by intracellular kinases to its active metabolite, lamivudine triphosphate (3TC-TP). This active form competes with the natural nucleotide deoxycytidine triphosphate for incorporation into viral DNA. Once incorporated, it acts as a chain terminator, preventing the elongation of viral DNA and thereby inhibiting replication [5,6]. The chemical structure of the phosphorylated metabolite is shown in Fig. 2.

Tenofovir, chemically known as [(R)-9-(2-phosphonomethoxypropyl) adenine], is classified as a nucleotide analogue reverse transcriptase inhibitor. It mimics natural nucleotides and, once phosphorylated intracellularly to its active form, tenofovir diphosphate competes with deoxyadenosine 5′-triphosphate for incorporation into viral DNA [6,7] by reverse transcriptase, as shown in Fig. 3.

A review of the existing literature reveals that only a limited number of analytical methods have been reported for the individual or simultaneous quantification of doravirine, lamivudine, and tenofovir. Particularly, few studies have explored their simultaneous estimation using advanced techniques such as high-performance liquid chromatography (HPLC), high-performance thin layer chromatography, or ultra-HPLC [8-10].

In view of this, the present study aimed to develop a fast, accurate, and reliable HPLC method for the simultaneous determination of these antiretroviral agents. The method was designed with green chemistry principles in mind, employing a less toxic mobile phase. In addition, the method requires minimal sample preparation, has a short chromatographic run time, and produces low solvent waste, making it both eco-friendly and cost-effective.

METHODS

Required instruments

Reverse-phase HPLC (RP-HPLC) analysis was carried out using a Waters Alliance 2695 HPLC system, equipped with a 2998 photodiode array detector. The system was controlled, and data were processed using Empower 2 software. Chromatographic separation was achieved on a ZORBAX Eclipse Plus C18 column (150 mm×2.1 mm, 5 µm particle size), offering high resolution and reproducibility. Additional laboratory equipment used during the study included an FS 4 ultrasonic bath sonicator (Mumbai, India) for sample degassing and dissolution, a Denver analytical balance for accurate weighing, and Whatman No. 41 filter paper for sample filtration before injection [10-12].

Chemicals and reagents

Doravirine, lamivudine, and tenofovir were obtained as gift samples from Hetero Drugs Ltd., Hyderabad, India. HPLC-grade acetonitrile was procured from SD Fine Chemicals, India. Milli-Q grade water was used throughout the analysis. Orthophosphoric acid and potassium dihydrogen phosphate (HPLC grade) were purchased from Merck Ltd., India.

Mobile phase preparation

To prepare the buffer solution, 1.36 g of dihydrogen potassium phosphate was accurately weighed and dissolved in approximately 900 mL of Milli-Q water in a 1000 mL volumetric spray bottle. The solution was thoroughly mixed, and the final volume was adjusted to 1000 mL with Milli-Q water. The pH was adjusted to 3.5 using 0.1% orthophosphoric acid. The prepared buffer solution was subsequently filtered through a 0.45 µm membrane filter. For the mobile phase preparation, the buffer was mixed with HPLC-grade acetonitrile in a 70:30 (v/v) ratio.

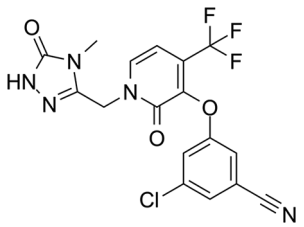

Fig. 1: Structure of doravirine

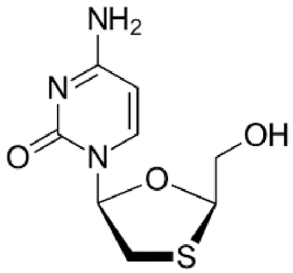

Fig. 2: Structure of lamivudine

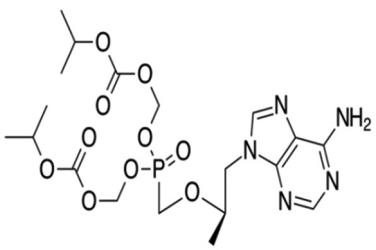

Fig. 3: Structure of tenofovir

Diluent selection

Based on the solubility profiles of the analytes, an appropriate diluent was prepared. Initially, the drugs were dissolved in methanol and then diluted with a mixture of acetonitrile and water in a 50:25:25 ratio (Methanol: Water: Acetonitrile).

Preparation of standard stock solutions

Weighed quantities of 5 mg of doravirine, 15 mg of lamivudine, and 15 mg of tenofovir were individually transferred into separate 50 mL volumetric flasks. Each drug was dissolved using a small volume of the prepared diluent and subjected to sonication for 20 min to ensure complete dissolution. Following this, the volumes were brought up to the mark with the same diluent. This resulted in stock solutions with final concentrations of 100 µg/mL for doravirine and 300 µg/mL each for lamivudine and tenofovir.

Preparation of standard working solutions

From the prepared standard stock solution, 1 mL was pipetted into a 10 mL volumetric flask and diluted to volume using a methanol–water mixture. This dilution yielded working standard concentrations of 10 µg/mL for doravirine and 30 µg/mL each for lamivudine and tenofovir.

Preparation of sample stock solutions

To determine the average tablet weight, five tablets were accurately weighed, and the mean weight was calculated. An amount equivalent to the average tablet weight was transferred into a 250 mL volumetric flask. To facilitate extraction, 25 mL of an appropriate diluent was added, and the mixture was shaken or sonicated for 50 min. The volume was then made up to 250 mL with the same diluent, and the solution was subsequently filtered for analysis.

Preparation of sample working solutions

A 0.25 mL aliquot of the filtered stock solution was transferred into a 10 mL volumetric flask and diluted to volume with the selected diluent. This preparation resulted in working concentrations of 10 µg/mL for doravirine and 30 µg/mL each for lamivudine and tenofovir.

Standard and sample solutions for assay analysis

Ten microliter aliquots, containing 10 µg/mL of doravirine, 30 µg/mL of lamivudine, and 30 µg/mL of tenofovir, from both the standard and sample solutions were injected 6 times into the HPLC system. The peak areas for each analyte were recorded, and the assay percentage was calculated by comparing the peak areas of the sample solutions with those of the corresponding standard solutions, as presented in Tables 1-3.

Assay results

The assay was performed using the marketed Delstrigo formulation, which contains doravirine (100 mg), lamivudine (300 mg), and tenofovir (300 mg). The average assay results obtained were 99.79% for doravirine, 100.07% for lamivudine, and 99.73% for tenofovir. These results are presented in Table 4.

Validation

System suitability parameters

To assess system performance, standard solutions containing 10 µg/mL of doravirine, 30 µg/mL of lamivudine, and 30 µg/mL of

Table 1: Results for assay studies

Drug	Delstrigo® tablet lable claim (mg/tablet)	Amount found* (mg/tablet)	Label claim %	RSD%
Doravarine	100	99.79	99.79	0.6
Lamivudine	300	300.21	100.07	0.3
Tenofovir	300	299.19	99.73	0.5

*Mean of six determinations. RSD: Relative standard deviation

Table 2: System suitability parameters

S. No.	Doravirine			Lamivudine			Tenofovir			Resolution
Inj	RT (min)	TP	Tailing	RT (min)	TP	Tailing	RT (min)	TP	Tailing	
1	2.195	2118	1.22	2.715	2932	1.28	3.660	3870	1.30	4.3
2	2.217	2581	1.31	2.718	2893	1.30	3.676	3977	1.28	4.4
2	2.224	2287	1.15	2.727	3245	1.31	3.682	4170	1.29	4.4
4	2.238	2022	1.20	2.753	3115	1.28	3.711	4141	1.28	4.4
5	2.238	2008	1.18	2.757	3116	1.29	3.720	4031	1.28	4.4
6	2.239	2076	1.17	2.761	2947	1.27	3.727	3861	1.27	4.3

Table 3: Linearity tables

Doravirine		Lamivudine		Tenofovir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
2.5	78463	7.5	227392	7.5	222215
5	164731	15	446498	15	448442
7.5	237096	22.5	656217	22.5	682527
10	314461	30	887260	30	907462
12.5	386326	37.5	1111671	37.5	1110557
15	466692	45	1332656	45	1338543

Table 4: System precision

Concentration (µg/mL)	Doravirine	Lamivudine	Tenofovir
	10	30	30
Area	274270±2065.4	8943233±3200.0	916455±3360.8
%RSD	0.8	0.4	0.4

All values are expressed as Mean±SD, *n*=6. RSD: Relative standard deviation

tenofovir were prepared and injected 6 times into the HPLC system. Key chromatographic parameters such as tailing factor, resolution, and the number of theoretical plates (USP) were evaluated. The method met the predefined system suitability acceptance criteria. The %RSD of peak areas for six replicate injections was found to be within 2%, confirming the system suitability and consistency of the method.

Specificity

To confirm the specificity of the developed method, both blank and placebo samples were analyzed. No interfering peaks were observed at the retention times corresponding to doravirine, lamivudine, or tenofovir, indicating that the method is specific to the analytes of interest and free from interference by excipients or other components.

Precision

Sample stock solution preparation

To determine the average tablet weight, five tablets were accurately weighed, and the mean weight was calculated. A quantity of powder equivalent to the average tablet weight was transferred into a 250 mL volumetric flask. To facilitate complete dissolution, 25 mL of diluent was added and the mixture was sonicated for 50 min. The volume was then made up to 250 mL with the same diluent followed by filtration. The resulting solution contained doravirine at 400 µg/mL and lamivudine and tenofovir each at 1200 µg/mL

Sample working solution preparation

Using a 10 mL volumetric pipette, 0.25 mL of the filtered standard solution was accurately transferred into a 10 mL volumetric flask and diluted to volume with the appropriate diluent. This resulted in working concentrations of 10 µg/mL for doravirine and 30 µg/mL each for lamivudine and tenofovir. To assess precision, six replicate injections of the prepared working solution were analyzed. The %RSD of the peak areas for each analyte was calculated and found to be within the acceptable limit of not more than 2%, demonstrating good repeatability and precision of the method.

Linearity

Standard stock solutions were prepared by accurately weighing 5 mg of doravirine, 15 mg of lamivudine, and 15 mg of tenofovir, and transferring each into separate 50 mL volumetric flasks. Approximately 50 mL of diluent was added to each flask, and the contents were sonicated for 20 min to ensure complete dissolution. The volumes were then made up to 50 mL with the same diluent, resulting in stock concentrations of 100 µg/mL for doravirine and 300 µg/mL each for lamivudine and tenofovir. To establish linearity, a series of standard solutions were prepared by pipetting varying volumes from the stock solutions and diluting to 10 mL with the same diluent. The resulting concentrations for doravirine ranged from 2.5 to 15 µg/mL, while those for lamivudine and tenofovir ranged from 7.5 to 45 µg/mL. These linearity solutions were used to evaluate the method's ability to produce accurate and proportional responses over the tested concentration range.

Accuracy

The sample stock solution was prepared by weighing five tablets to determine the average weight, after which a portion equivalent to one tablet was transferred to a 250 mL volumetric flask. Twenty-five milliliters of diluent was added, and the mixture was sonicated for 50 min. The volume was then made up with diluent and filtered to obtain a solution containing 400 µg/mL of doravirine and 1200 µg/mL each of lamivudine and tenofovir. A standard working solution was prepared by pipetting 1.0 mL from the standard stock solutions (100 µg/mL doravirine, 300 µg/mL lamivudine, and 300 µg/mL tenofovir) into a 10 mL volumetric flask and diluting to volume, yielding 10 µg/mL doravirine and 30 µg/mL of lamivudine and tenofovir.

For spiked recovery solutions, 0.5 mL, 1.0 mL, and 1.5 mL of the sample stock were taken for the 50%, 100%, and 150% levels, respectively, each spiked with 1.0 mL from the standard stock solutions and made up to 10 mL with diluent.

Robustness

To evaluate the robustness of the method, deliberate minor variations were introduced in key analytical parameters, including flow rate, mobile phase composition, and column temperature. The flow rate was adjusted to 0.7 mL/min and 0.9 mL/min; the mobile phase ratio was modified to 65:35 and 75:25 (v/v); and the column temperature was altered to 21.0°C and 31.0°C. The results showed consistent system suitability parameters and %RSD values within the acceptable limits, thereby confirming the robustness of the method as per International Council for Harmonization (ICH) guidelines.

Limits of detection (LOD) sample preparation

To prepare samples for determining the LOD, 0.25 mL of each standard stock solution (Doravirine, Lamivudine, and Tenofovir) was individually transferred into separate 10 mL volumetric flasks and diluted to volume with the appropriate diluent. Similarly, for the limit of quantification (LOQ), 0.3 mL of each solution was transferred into individual 10 mL volumetric flasks and diluted to the mark using the same diluent.

LOQ sample preparation

To determine the LOQ, 0.25 mL of each standard stock solution (Doravirine, Lamivudine, and Tenofovir) was accurately transferred into separate 10 mL volumetric flasks and diluted to volume with the appropriate diluent. In addition, 0.3 mL of each standard solution was similarly transferred into separate 10 mL flasks and diluted to the mark, to further support quantification and ensure reproducibility at low concentration levels

Degradation studies

Forced degradation studies were conducted to evaluate the stability of doravirine, lamivudine, and tenofovir under various stress conditions including oxidative, acidic, alkaline, thermal, photolytic, and neutral environments.

Oxidative degradation was induced by adding 1 mL of 20% hydrogen peroxide to 1 mL of stock solution of each drug and incubating at 60°C for 30 min, followed by dilution to 15 µg/mL and HPLC analysis. Acidic degradation involved mixing 1 mL of drug solution with 1 mL of 2 N HCl, incubating at 60°C for 30 min, neutralizing, and diluting to final concentrations of 10 µg/mL for doravirine and 15 µg/mL each for lamivudine and tenofovir before injection into the HPLC system. Alkaline degradation was carried out similarly using 2 N NaOH under the same thermal conditions, with subsequent neutralization and dilution. For thermal degradation, drug solutions were exposed to dry heat at 105°C for 1 h and then diluted to respective final concentrations for HPLC analysis. Photostability was assessed by exposing 100 µg/mL of doravirine and 300 µg/mL each of lamivudine and tenofovir to Ultraviolet (UV) light for 24 h (approx. 200 h/m^2), followed by dilution to 10 µg/mL and 15 µg/mL, respectively, and chromatographic analysis. Neutral degradation was evaluated by refluxing the drug solutions in water at 60°C for 6 h, followed by appropriate dilution and injection into the HPLC system. Chromatograms from all stress conditions were examined to determine the stability and degradation profiles of the analytes.

RESULTS AND DISCUSSION

Method development

The optimized chromatographic conditions consisted of a mobile phase composed of 0.01 N potassium dihydrogen phosphate and acetonitrile in a 70:30 (v/v) ratio, delivered at a flow rate of 1.0 mL/min. Separation was carried out on a ZORBAX C18 column (2.1×150 mm, 5 µm particle size) with the column temperature maintained at 30°C. Detection was performed at a wavelength of 244.0 nm, with an injection volume of 10 µL and a total run time of 5.0 min. Under these conditions, all three analytes were eluted with satisfactory retention times and resolution, and all system suitability parameters – such as tailing factor and theoretical plate count – were within acceptable limits as per ICH guidelines (Fig. 4).

Method validation

System suitability

The system suitability parameters for doravirine, lamivudine, and tenofovir are summarized in Table 2, which demonstrates that all values fall within the acceptable range as per ICH guidelines. Parameters such as retention time, tailing factor, theoretical plates, and resolution confirm the adequacy of the chromatographic system for simultaneous analysis.

Specificity

Doravirine, lamivudine, and tenofovir exhibited well-resolved retention times of 2.254, 2.799, and 3.812 min, respectively. No interfering or extraneous peaks were observed at the retention times of these analytes in either blank or placebo chromatograms, indicating the absence of matrix interference.

Linearity

Table 5 presents the linearity data for doravirine, lamivudine, and tenofovir over their respective concentration ranges. The method exhibited excellent linearity with correlation coefficients (R^2) close to 1.000, indicating strong linear relationships between concentration and peak area for all three analytes.

Figs.5-7 showed the calibration curves for doravirine, lamivudine and tenofovir, respectively, which further illustrate the linear response of the method across the tested range.

Precision

The precision of the developed RP-HPLC method was evaluated in terms of system precision, repeatability, and intermediate precision, in accordance with ICH Q2 (R1) guidelines. All results confirm the method's high degree of precision, suitable for routine quality control analysis, as shown in Tables 4-6.

Accuracy

The accuracy of the developed RP-HPLC method was evaluated through recovery studies conducted at three concentration levels – 50%, 100%, and 150% – for doravirine, lamivudine, and tenofovir. For doravirine, the percentage recovery ranged from 99.3% to 100.8%, with a mean recovery of 100.02%, indicating excellent accuracy. Lamivudine showed recovery values between 99.62% and 100.26%, with a mean of

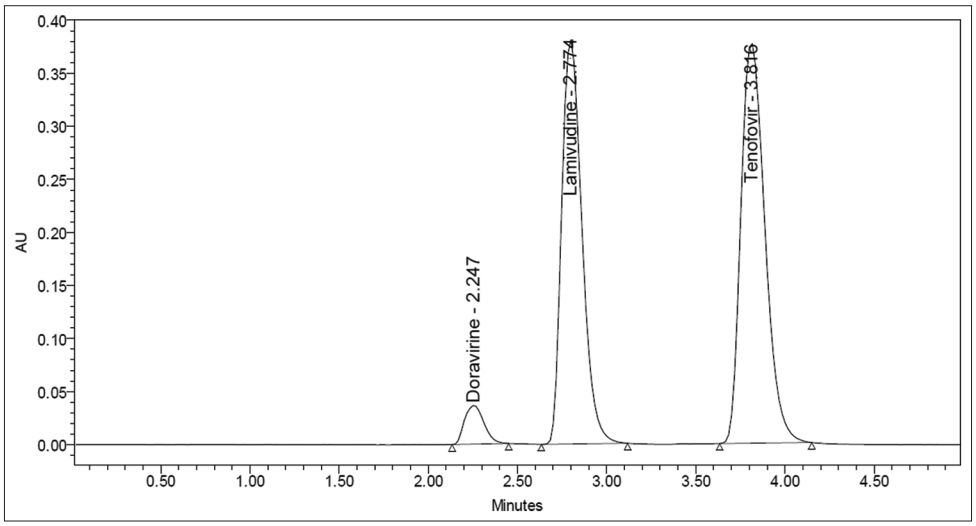

Fig. 4: Optimized chromatogram

Table 5: Intermidiate precission

Concentration (µg/mL)	Doravirine	Lamivudine	Tenofovir
	10	30	30
Area	287146±4411.0	897289±6647.2	914043±3444.9
%RSD	1.5	0.7	0.4

All values are expressed as Mean±SD, *n*=6. RSD: Relative standard deviation

Table 6: Repeatability

Concentration (µg/mL)	Doravirine	Lamivudine	Tenofovir
	10	30	30
Area	274255±1710.2	896752±2494.5	915770±4455.4
%RSD	0.6	0.3	0.5

All values are expressed as Mean±SD, *n*=6. RSD: Relative standard deviation

100.09%, further confirming the method's precision in quantifying this analyte. Tenofovir exhibited recovery in the range of 99.43–100.15%, with a mean recovery of 99.98%. All results fall within the acceptable limits as per ICH guidelines, as shown in Table 7.

Sensitivity

The sensitivity of the proposed RP-HPLC method was determined by evaluating the LOD and LOQ for doravirine, lamivudine, and tenofovir. Doravirine exhibited the highest sensitivity, with a LOD of 0.01 µg/mL and a LOQ of 0.04 µg/mL. Lamivudine and tenofovir both had a LOD of 0.11 µg/mL, with corresponding LOQ values of 0.32 µg/mL and 0.34 µg/mL, respectively. These results indicate that the method is capable of detecting and quantifying very low concentrations of the analytes, making it suitable for routine analysis and trace-level detection in pharmaceutical formulations, as shown in Table 8.

Robustness

As shown in Table 9, the %RSD values for doravirine, lamivudine, and tenofovir remained below 2.0% under all tested conditions, indicating that the method is robust and capable of withstanding minor changes without significant impact on the results. These findings confirm the method's reliability for routine use in varied laboratory environments.

Degradation

The drug was subjected to forced degradation under stress conditions, including acid, alkali, oxidative, thermal, UV, and aqueous environments, to evaluate its stability. All degraded samples were analyzed chromatographically, and the results confirmed that degradation remained within acceptable limits for all conditions. The drug showed higher sensitivity under acidic conditions, while moderate degradation was observed under alkaline and oxidative stress. Minimal degradation occurred under thermal, UV, and water stress, indicating good stability. Chromatographic profiles for each condition are presented in Table 10.

Greenness assessment of the developed method

In analytical chemistry, greenness assessment focuses on developing sustainable methods with minimal environmental impact. Factors such as solvent selection, energy consumption, waste generation, and safety play a central role. While the current RP-HPLC method employs acetonitrile – a commonly used organic solvent with known environmental concerns – the method remains green in other critical aspects. By optimizing chromatographic conditions, minimizing solvent volume, avoiding derivatization, and reducing waste, the overall ecological footprint of the procedure is substantially lowered. Moreover, proper waste handling and adherence to safety protocols enhance the method's environmental responsibility. The AGREE evaluation tool was used to assess the method's alignment with the twelve principles of green analytical chemistry (GAC). The tool provides a visual pictogram and a numerical score ranging from 0 to 1, where higher values indicate greater environmental compliance. In this study, the developed method for simultaneous estimation of doravirine, lamivudine, and tenofovir achieved an AGREE score of 0.73, reflecting good environmental

Table 7: Accuracy table

Drug	% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
Doravirine	50%	5	4.965	99.3	100.02%
		5	4.996	99.9	
		5	5.044	100.9	
	100%	10	10.008	100.1	
		10	10.084	100.8	
		10	9.927	99.3	
	150%	15	14.950	99.7	
		15	14.927	99.5	
		15	15.111	100.7	
Lamivudine	50%	15	14.95	99.69	100.09%
		15	14.99	99.92	
		15	15.04	100.26	
	100%	30	30.28	100.93	
		30	30.01	100.04	
		30	29.94	99.80	
	150%	45	44.82	99.59	
		45	45.24	100.53	
		45	45.04	100.09	
Tenofovir	50%	15	15.02	100.15	99.98%
		15	14.87	99.13	
		15	14.98	99.88	
	100%	30	30.14	100.46	
		30	29.99	99.95	
		30	30.03	100.11	
	150%	45	45.43	100.95	
		45	44.99	99.98	
		45	44.64	99.20	

Table 8: Sensitivities of doravirine, lamivudine, and tenofovir

Drugs	LOD (µg/mL)	LOQ (µg/mL)
Doravirine	0.01 µg/mL	0.04 µg/mL
Lamivudine	0.11 µg/mL	0.32 µg/mL
Tenofovir	0.11 µg/mL	0.34 µg/mL

LOD: Limits of detection, LOQ: Limit of quantification

Table 9: Robustness data for doravirine, lamivudine, and tenofovir

S. No.	Condition	%RSD of doravirine	%RSD of lamivudine	%RSD of tenofovir
1	Flow rate (−) 0.7 mL/min	0.7	0.5	0.5
2	Flow rate (+) 0.9 mL/min	0.5	0.5	0.6
3	Mobile phase (−) 65B: 35A	1	0.3	1.5
4	Mobile phase (+) 75B: 25A	1.1	0.6	0.2
5	Temperature (−) 21°C	0.4	0.1	0.1
6	Temperature (+) 31°C	1	0.1	0.7

RSD: Relative standard deviation

Table 10: Degradation study data

S. No.	Degradation condition	% Drug undegraded	% Drug degraded
1	Acid	92.62	7.38
2	Alkali	97.99	2.01
3	Oxidation	96.42	3.58
4	Thermal	98.16	1.84
5	UV	99.18	0.82
6	Water	98.92	1.08

RSD: Relative standard deviation, UV: Ultraviolet

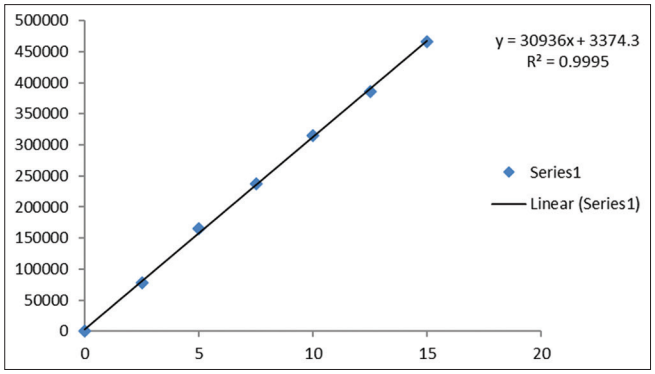

Fig. 5: Calibration curve doravirine

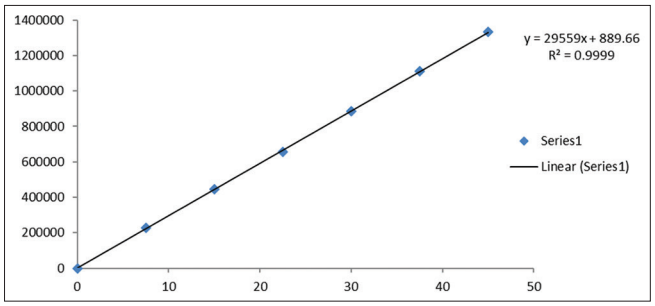

Fig. 6: Calibration curve lamivudine

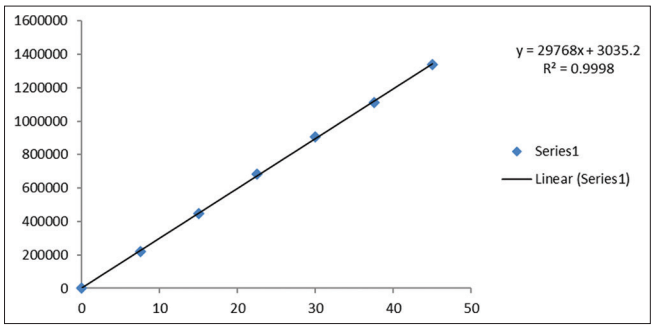

Fig. 7: Calibration curve tenofovir

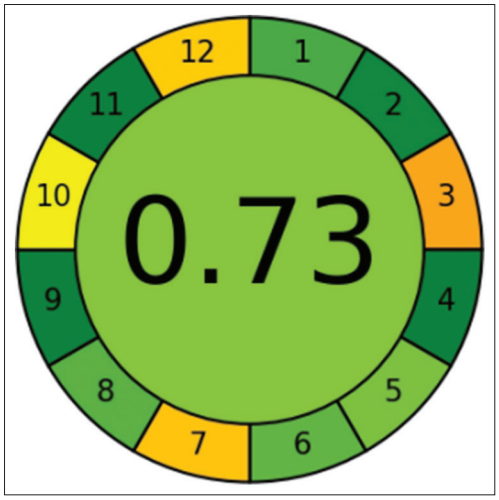

Fig. 8: Green assessment by AGREE tool

compatibility despite the use of acetonitrile. The AGREE pictogram, presented in Fig. 8, confirms the method's suitability for routine pharmaceutical analysis within a sustainable framework.

CONCLUSION

A simple, robust, and reliable RP-HPLC method was successfully developed and validated for the simultaneous quantification of doravirine, lamivudine, and tenofovir in fixed-dose combination tablets. The method demonstrated excellent system suitability, precision, and accuracy, with low %RSD values and recovery rates close to 100%, confirming its reliability. Sensitivity was validated by low LOD and LOQ values, ensuring suitability for trace-level analysis. Linearity was established over a broad concentration range with strong correlation coefficients and regression equations. Reduced retention times contributed to high sample throughput and reduced solvent consumption, enhancing both time and cost efficiency. Although acetonitrile was used as the organic modifier, the method achieved an AGREE score of 0.73, reflecting good alignment with GAC principles. In addition, the drug was subjected to forced degradation under various stress conditions – acidic, alkaline, oxidative, thermal, UV, and aqueous – to evaluate its stability. Chromatographic analysis confirmed that degradation in all conditions remained within acceptable limits. Overall, the method complies with ICH Q2 (R1) guidelines and is suitable for routine quality control, batch release, stability testing, and environmental sustainability considerations in the pharmaceutical industry.

AUTHORS CONTRIBUTIONS

Sirisha Gorantla: Planning, conceptualization, data collection, and paperwriting. Subhranshu Panda: Review of literature and data interpretation. All the authors have read and agreed to publish the manuscript.

CONFLICTS OF INTEREST

Not applicable.

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