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Original Article

Quantitative Evaluation of White Analytical Chemistry Metrics in Method Development and Validation for the Simultaneous Determination of Antiretroviral Drugs

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ABSTRACT

Background: Lamivudine, Dolutegravir, and Tenofovir alafenamide are widely used antiretroviral agents in the treatment of human immunodeficiency virus-1infection. Accurate and sustainable analytical methods are essential for their quality control in combination dosage forms. **Objectives:** The study aimed to develop and validate a novel, and environmentally sustainable reverse-phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous quantification of Lamivudine, Dolutegravir and Tenofovir alafenamide in tablet-dosage forms. **Materials and Methods:** Chromatographic separation was performed using a 70:30 (v/v) methanol-buffer as a mobile phase at 0.7 mL/min, 25°C, with detection at 264 nm. Method validation followed ICH-guidelines for linearity, accuracy, robustness, and sensitivity. **Results:** The retention times for Lamivudine, Tenofovir alafenamide and Dolutegravir were 3.142, 4.612, and 6.792 min, with (%RSD) relative standard deviation of 0.2, 0.5, and 0.08, and recoveries of LMU, DTG and TA for 80% was found to be 100.2%, 99.81% and 99.49 respectively, for 100% was found to be 99.51%, 99.30% and 99.39% and for 120% was found to be 99.77%, 99.21% and 100.4% respectively. The method showed linearity for Lamivudine and Tenofovir alafenamide (12-60 µg/mL; $R^2 > 0.9999$ and $R^2 > 0.9997$ respectively) and Dolutegravir (2–10 µg/mL; $R^2 > 0.9994$), with limit of detection of 0.12, 0.24, and 0.21 µg/mL and limit of quantification of 0.36, 0.73 and 0.34 µg/mL, respectively. Forced–degradation studies confirmed method's the stability-indicating ability. Notably, the method showed superior eco-performance, using less than 30 mL of solvents versus 100 mL in the proposed method, reducing waste and environmental impact. Its greenness was validated by higher score in AGREE (0.77), Mo-GAPI (78) and AGSA (76). The present RP-HPLC method achieved a whiteness score of 85.33 (avg. of 90+76+90) across analytical efficiency and cost-time metrics. **Conclusion:** Proposed RP-HPLC method is cost-effectiveness, speed, and environmental sustainability for the simultaneous quantification of three drugs combinations.

Keywords: Dolutegravir, Lamivudine, RP-HPLC, Tenofovir alafenamide, White Analytical Chemistry.

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INTRODUCTION

Lamivudine is a reverse transcriptase that has been clinically reported to be helpful against the hepatitis B, HIV-1, and HIV-2 viruses.[1] It improves liver histology stages and the development of seroconversion to antigen in beneficial hepatitis B.[2] Human Immunodeficiency Virus (HIV) is a retrovirus that primarily attacks the immune system by targeting CD4 T cells. The treatment of HIV/ AIDS relies on antiretroviral therapy, which includes a combination of medicines that inhibit viral replication and reduce the viral load, thereby improving patient survival and quality of life. LMU is chemically 4-amino-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-2(1H)-pyrimidinone; it is a Nucleoside Reverse Transcriptase Inhibitor (NRTI) that interferes with the viral replication process by inhibiting the activity of the reverse transcriptase enzyme.[3] Tenofovir Alafenamide is also classified as an NRTI it undergoes intracellular conversion to its active metabolite, which competitively inhibits viral reverse transcriptase, leading to unseasonable termination of viral DNA extension. TA is chemically isopropyl (2S)-2[[[(1R)-2-(6-aminopurin-9yl)-1-methyl-ethoxy]-methyl-phynoxy-phosphoryl]-amino]-propanoate used as an HIV.[4] Dolutegravir is an integrase inhibitor that prevent the integration of viral DNA into the host genome. It does this by chelating magnesium ions in the active site of the HIV

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integrase enzyme, thereby blocking the beachfront transfer step necessary for viral replication, DTG is highly effective in rapidly lowering viral load while simultaneously boosting the number of CD4 cells in patients treated with 10, 25, or 50 mg once daily, in addition to a nucleoside reverse transcriptase inhibitor (NRTI) background. DTG is a chemically (4*R*,12a*S*)-*N*-[(2,4-difluorophenyl) methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido [1′,2′:4,5] pyrazino[2,1-*b*][1–3] oxazine-9-carboxamide. As mentioned in the literature, several approaches are practical for predicting TA, LMU and DTG individually. Among these, LMU, TA and DTG are extensively used antiretroviral agents with distinct mechanisms of action that contribute to effective HIV operation.[5]

LMU, DTG and TA in pharmaceutical dosage forms cannot be simultaneously estimated through a simple and rapid analytical process by using assessment of white analytical chemistry as indicated by the literature review. Furthermore, no statistical method for evaluating combinations of LMU, DTG and TA was identified through our conventional prior-art search. Multiple research studies that describe research they conducted do, however, include methodological flaws, such as the use of an ion-pair reagent in preparing the mobile phase and a run time surpassing 12 min. Additionally, photo-stable, the temperature and water conditions are not utilized for forced degradation. In the literature search, limited RP-HPLC stability-indicating assay method has been previously reported for the simultaneous quantification of all three drugs. In the present study, we discuss the development and validation of a simple, precise, accurate, and inexpensive RP-HPLC stability-indicating assay method for quantifying LMU, DTG and TA in pharmaceutical dosage forms by white analytical chemistry using green assessment tools. Based on a review of literature of all drugs are individual and combine a few methods that have been developed by [UV],[6] [HPTLC],[7–8][RP-HPLC],[9–11] and bioanalytical.[12]

The objectives were to reduce or eliminate operations that generate hazardous materials, which pose serious threats to the environment and public health. The 12 principles of green chemistry, also known as "productively," were introduced by the same researchers who developed the concept of implementing green chemistry. The development of novel techniques and protocols involves carefully selecting the right solvents, materials, and technologies to regulate the safety and reactivity of chemical processes. The aim of this study is to improving method through green chemistry to reduce toxic waste through material reuse, energy conservation and process efficiency enhancements.[13] Green analytical techniques have emerged as a result of this new idea; they are faster than standard methods, use fewer chemicals and solvents, and generate less waste, all of which reduce toxicity. In addition to green chemistry principles, Galuszka *et al.* introduced 12 green analytical principles under the acronym "significance," focusing on chemometric data use and miniaturized systems.[14]

White Analytical Chemistry (WAC) is an evolution of Green Analytical Chemistry (GAC), condensing its 12 principles into four key green principles that highlight the core aspects of GAC. WAC evaluates method quality by integrating analytical, practical, ecological and functional factors, using the green, red and blue color model to define a "white" (balanced) method.[15–17] The red group assesses the validation characteristics of the analytical method, including sensitivity, accuracy, precision and scope. The green group focuses on GAC-related environmental safety factors, including reagent toxicity, reagent quantity and type, waste production, energy utilization and overall environmental impact. The blue group focuses on operational aspects, including cost, time efficiency, practical requirements and simplicity. A score of 100 is fully satisfactory, 75 is excellent but not universally suitable and 50 is occasionally acceptable. Averages are based on parameter scores in green, red and blue categories.[18–19]

MATERIALS AND METHODS

Chemicals and reagents

The Active Pharmaceutical Ingredients (API standards) sample of Lamivudine 99%, Dolutegravir 99.5% and Tenofovir alafenamide 99.3% were provided from Mylon Pharmaceuticals in Hyderabad, India, as a gift sample. A tablet Acriptega containing 300 mg Lamivudine, 300 mg Tenofovir alafenamide and 50 mg Dolutegravir, in a fixed dosage combination, was purchased from a local market in Nagpur, India.

All reagents utilized in this study were of HPLC grade and were sourced from SD Fine-Chem, Mumbai. The HPLC analytical methods were conducted using Milli-Q water, acetonitrile 99.8%, methanol 99.8% as the respective solvents. Triethyl amine 99% Extra pure, Hydrochloric acid 35 to 38% AR grade sd Fine, Sodium hydroxide 98% AR grade (SRL) Hydrogen peroxide 6% w/v Loba chemie Pvt. Ltd., ortho phosphoric acid 85% AR grade SRL.

Instrumentation

Agilent 1100 HPLC consisting of a pump G1310A, ISO auto sampler, auto injector, DAD detector (Model G13148), thermostatic column compartment connected with Chem Station software (Agilent Technologies, USA), associated with a Agilent Zorbax Eclipse C18 (250×4.6 mm, 5µ) Column. Shimadzu analytical balance (Model AY220, Japan) for accurate weighing, a Systronics digital pH meter (Model µ 361, Ahmedabad, India) for pH adjustment, and a Spectra Lab ultrasonic bath Sonicator (India).

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Mobile phase preparation (Preparation of 0.05% triethylamine buffer solution)

TEA (0.05mL) was measured accurately by pipetting into 1000 mL Mili Q water and dissolved. The pH was adjusted to 3.0 with dilute with ortho phosphoric acid.

Standard solution preparation

Accurately weighed quantity of API powders equivalent to 60 mg of LMU, 60 mg of TA and 10 mg of DTG were taken into a 25 mL of volumetric flask and methanol was added to dissolve the contents, followed by the addition of methanol to make up the final volume of concentration 2400 µg/mL of LMU, 2400 µg/mL of TA and 400 µg/mL solutions respectively. Further, 0.05 mL of the stock solution was pipette out into 10 mL volumetric flask and final volume was made up with methanol to obtain final concentration of 12ppm for LMU, 12ppm for TA and 2 ppm for DTG working solution. The solution was filtered through a 0.22 µm membrane filter before analysis.

Tablet Sample preparation

Twenty tablets were weighed, crushed, and an amount of tablet powder 276.6 mg equivalent to 60 mg of LMU, 60 mg of TA and 10 mg of DTG was transferred

to 25 mL volumetric flask. Methanol was added to dissolve it, sonicated for 10 min and the final volume was made up with methanol. Further, 0.05 mL of the stock solution was pipette out into 10 mL volumetric flask and final volume was made up with methanol to obtain final concentration of 12ppm for LMU, 12ppm for TA and 2 ppm for DTG sample solution. The solution was filtered through a 0.22 µm membrane filter before analysis.

EXPERIMENTAL WORK

System suitability

Twenty microliters of the working solutions (12 µg/mL for LMU, 12 µg/mL for TA and 2 µg/mL for DTG) were injected. The system suitability parameters were determined by making six replicate injections and analyzing each solute for its retention time, tailing factor (T) and theoretical plate number (N). A chromatogram for the LMU, DTG and TA standard was illustrated in (Figure 1).

Accuracy

Recovery studies were conducted to verify the accuracy of the proposed method at concentration 24, 24 and 4 µg/mL LMU, TA and DTG respectively. Known quantity of standard solution at three distinct concentrations (80%, 100%, and 120%) was spiked and then calculated the recovery using area of chromatogram.

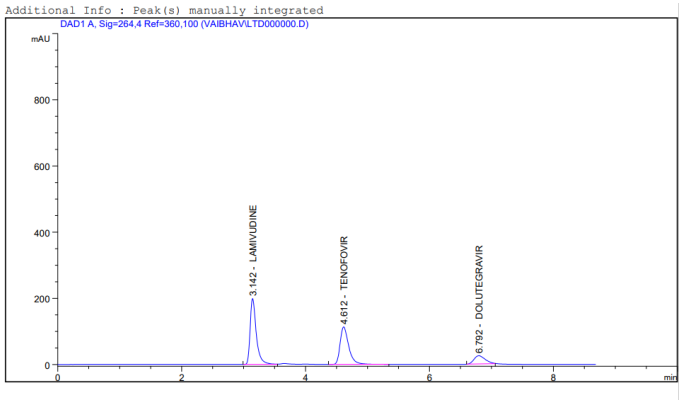


Figure 1: Standard chromatogram of lamivudine, tenofovir alafenamide, and dolutegravir.

Precision

Six replicate injections of working solution (12, 12, and 2 µg/mL of LMU, TA, and DTG respectively) were used to determine precision at both intraday and inter-day levels.

Linearity and range (R^2 Value)

Pipette out 0.05, 0.1, 0.15, 0.2 and 0.25 mL of standard stock solution of LMU, TA and DTG in 10 mL of volumetric flask separately and made up the volume to obtained the various concentrations of (12, 24, 36, 48, 60 µg/mL LMU and TA) and (2, 4, 6, 8, 10 µg/mL DTG) respectively.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The least amount of analyte in a sample that can be identified is known as the Limit of Detection (LOD) for a method. The lowest analyte concentration in a sample that can be determined with a sufficient degree of precision and accuracy under the specified operating conditions of the method is known as the Limit of Quantitation (LOQ).

The standard deviation of the response (SD) and the slope of the calibration curve(s) at values near the LOD can be used to calculate LOD and LOQ using the following formulas:

$$LOD = 3.3 \times S/SD \text{ and } LOQ = 10 \times S/SD$$

Robustness

Robustness parameter was assessed at slight and deliberate adjustments, such as changes in flow rate (0.5 and 0.9 mL/min), mobile phase composition (65% Methanol: 35% Buffer and 75% Methanol: 25% Buffer) and changes in wavelength (262 nm and 266 nm). The robustness was examined using 12 µg/mL for LMU, 12 µg/mL for TA and 2 µg/mL for DTG in triplicate.

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Standard solution stability

At room temperature, the stability of the standard solution was tested every 24 and 48 hr. The system's appropriate characteristics, including theoretical plates, tailing factors, retention time and resolution were recorded.

Mobile phase stability

At ambient temperature, the stability of the mobile phase was systematically evaluated at intervals of 24 and 48 hr. The parameters including peak areas, theoretical plates, tailing factors, retention time and resolution were recorded.

Analysis of the marketed sample

The optimized method was applied to determine the purity of marketed formulation (Acriptega). The tablet sample solution was suitably diluted to obtain various concentrations of 12 µg/mL for LMU, 12 µg/mL for TA and 2 µg/mL for DTG and injected into the chromatographic system. The corresponding peak areas were determined and assay values were calculated.

% Assay = Sample Peak Area/Standard peak area * Std. concentration/Sample concentration *100

Forced degradation study

Forced degradation study was conducted according to ICH guidelines to establish the stability-indicating nature of method. Standard stock solution of LMU (2400 µg/mL), TA (2400 µg/mL) and DTG (400 µg/mL) were used. For acid hydrolysis, 3 mL of 0.1 N HCl was added to the 1 mL of the above standard stock solution LMU, TA and DTG solutions in a round bottom flask and refluxed at 70°C for 1 hr the solution was neutralized with 0.1 N NaOH after acid hydrolysis then 0.05 mL were diluted with 10 mL methanol to obtained the desired concentration (12 µg/mL of LMU, 12 µg/mL TA and 2 µg/mL of DTG. Degraded sample were injected to the HPLC systems and compare the result with undegraded samples respectively. Similarly, alkali hydrolysis was performed using 0.1 N NaOH under the same conditions, followed by neutralization with 0.1 N HCl and subsequent analysis. Oxidative degradation, 1 mL of LMU, 1 mL of TA and 1 mL of DTG was treated with 3 mL of 3% hydrogen peroxide at RT for 1 hr then 0.05 mL of this solution was diluted with 10 mL of methanol and injected. For thermal degradation, 60 mg of LMU, 60 mg of TA and 10 mg of DTG were degraded in an oven then diluted in 25 mL of volumetric flask and make up the volume. Further 0.05 mL of above solution diluted to 10 mL of methanol and then injected. The neutral degradation, 3 mL of standard stock solution reflux with 3 mL of water for 2 hr. Then 0.05 mL of solution were diluted with 10 mL methanol and solution were injected. The degraded samples from each condition were analysed using HPLC and the chromatograms were compared with those of undegraded samples to identify and resolve any degradation peaks. The percentage of drug degraded under each condition was determined.

Statistical Analysis

The HPLC data is analyzed utilizing the Chem Station software. The outcomes were computed as the mean ± Standard Deviation (SD) to assess accuracy, while the Relative Standard Deviation (RSD) was determined to evaluate precision. Additionally, the regression coefficient was computed to ascertain the linearity parameter. AGREE software (an analytical greenness calculator version 0.5 beta) and MO-GAPI, BAGI (calculator version 1.4.3) and ASGA (version v0.37) software were used to examine the method's greenness profile.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

The approach to developing novel medicine combinations is unavoidable and always shows an advantage in treating illnesses. New techniques will always be needed to analyse the purity of the new medication combinations in the dosage form. In compliance with the updated ICH Q2(R^2) guidelines.[20]

However, rather than formulating an analytical approach using toxic solvents, if the method has developed based on eco-friendly solvents, then the developed method can be eco-friendly and sustainable. By removing hazardous chemicals, replacing them with more environmentally friendly alternatives, and eliminating waste production, the ecologically benign analysis will be feasible. According to the Environmental, Health and Safety (EHS) system, deleterious solvents like ACN, tetrahydrofuran, should avoid in LC separations. Instead, the employment of biodegradable solvents is advocated for the purpose of drug separation within LC methodologies. This current method development has structured such a way that mitigate adverse effects to both the analyst and the environment. However, numerous additional factors have been considered apart from the solvent when analysing the mixture's drugs. When evaluating fixed-dose combinations, it is crucial to keep in mind that the method should function for an extended period without requiring further validation. To eliminate all the impediments, the present study has been conducted using RP-HPLC, which enables the analysis of chemicals at low concentrations, to establish a green and sustainable approach for the future.

The ICH Q2(R^2) guidelines were followed in the validation of the developed method. The achievement of a satisfactory resolution between three drugs must be evaluated to develop a unique stability-indicating high-performance liquid chromatographic method for the quantification of LMU, TA and DTG.

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To achieve good separation between all three components, different buffer pH conditions and varying proportions of solvents, such as methanol and buffer were tested. Detection was also performed at a wavelength of 264 nm.

Finally, a mixture of methanol and buffer 0.05% Triethylamine in a 70:30 ratio achieved satisfactory results using isocratic elution on a C18 column (250 mm × 4.6 mm, 5 µm) and a flow rate of 0.7 mL/min and injection volume 20µL resulting in optimal chromatographic separation, in a continuous run of up to 10 min.

Validation of the developed method

Using ICH Q2(R^2) recommendations, the final optimized conditions were applied to the suggested RP-HPLC method and validated for criteria such as linearity, accuracy, and precision. Additionally, this method was used to determine the medications' % purity using the marketed formulation. Finally, various stressful conditions were used to conduct the forced deterioration study.

System Suitability

The system's suitability was tested to see how well it worked with recently made stock solutions. Comparing the Rt with those of standards allowed us to confirm the peak identities for LMU, DTG, and TA. The retention times for LMU, TA, and DTG are 3.142, 4.612, and 6.792 min, tailing factor mean are 0.621, 0.625 and 0.72 as well as theoretical plates are 5462, 5762 and 6241 respectively, as shown in (Table1). The system suitability parameters and resolution were reproducible and within the limits of the guidelines. The standard chromatograms for the three drugs are depicted in (Figure 1).

Accuracy

Accuracy (Trueness) was determined with the help of three different quality control concentration levels 80%, 100% and120%, for all the three drugs and the recovery percentage was found to be within limits as shown in Table 1. The results for the accuracy as the mean recovery of LMU, DTG and TA for 80% was found to be 100.2%, 99.81% and 99.49 respectively, for 100% was found to be 99.51%, 99.30% and 99.39% and for 120% was found to be 99.77%, 99.21% and 100.4%. Therefore, the given method shows satisfactory accuracy.

Precision

Intra-day precision for the investigated combination was assessed by calculating the percent Relative Standard Deviation (%RSD) after analyzing three freshly prepared solutions in six replicates of 12 µg/mL LMU, 12 µg/mL TA and 2 µg/mL on the same day. Intermediate precision the proposed method's inter-day accuracy was assessed by measuring six replicates over three days and calculating the percent Relative Standard Deviation (%RSD). The %RSD for intraday precision of LMU, TA and DTG was obtained as 0.3, 0.13 and 0.01 respectively and the %RSD for inter-day precision LMU, TA and DTG was obtained 0.31, 0.12 and 0.01 respectively. The %RSD of both the intraday and inter-day was less than 2%, indicating good precision of the proposed method as shown in Table 1.

Linearity and Sensitivities

Linearity can be measured at various analyte concentrations 12-60 µg/mL LMU, 12-60 µg/mL TA and, DTG 2-10 µg/mL respectively. Linear regression equations were y=123.45x-88.478 R^2=0.9999 for LMU, y=211.39x-112.23 R^2=0.9997 for DGT, and y=100.14x-201.99 R^2=0.9994 for TA. Adequate sensitivities for the developed method concerning the revealed LOD and LOQs were achieved. LODs and LOQs were (0.12µg/mL and 0.38µg/mL), (0.21µg/mL and 0.34µg/mL) and (0.24 µg/mL and 0.73µg/mL) for LMU, DTG and TA respectively. The results of both linearity and sensitivity in were depicted in Table 1.

Robustness

At flow rate of 0.5 and 0.9 mL/min the result for LMU % RSD was 0.13 0.46, DGT for 0.06, 0.04 and TA for 0.02, 0.30 respectively. The mobile phase (65% Methanol: 35% Buffer and 75% Methanol: 25% Buffer) % RSD were 0.11, 0.03 for LMU, 0.13, 0.11 for DTG and 0.005, 0.02 for TA respectively. At wavelength 262 nm it shows % RSD 0.35, 0.001, 0.14

for LMU, DTG and TA respectively and at wavelength 266 nm it shows % RSD 0.05, 0.033, 0.05 LMU, DTG and TA respectively. The standard deviation of the peak areas was calculated for each parameter and the %RSD was found to be less than 2%. The low values of the %RSD, as shown in Table 1, of three drugs, which indicated the method's robustness.

Analysis of the marketed sample

The method has been successfully applied for the analysis of a marketed tablet. The percent of drug content in tablet of LMU, DTG and TA was found to be 100.22%, 99.99%, 99.99%, respectively as well as %RSD of LMU, DTG and TA was found to be 0.075, 0.017 and 0.175 respectively. No interference from the additives has been observed, results are shown in Table 2.

Forced Degradation Studies

Drug combinations were assessed using various stressed conditions like acid, alkali, oxidation, thermal and neutral degradation.[21] Degradation chromatogram was represented in (Figure 2 a-e). The results are summarized as follows: LMU showed maximum degradation under acidic conditions (13.18%), followed by basic conditions (10.89%). The drug exhibited comparatively low degradation under oxidative (1.65%), thermal (1.69%), and neutral (2.29%) conditions. This indicates that Lamivudine is more susceptible to acid and base hydrolysis, suggesting the need for pH control during formulation and

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Table 1: Validation Parameter obtained for the developed method.

Sl. No.	Validation Parameter	Observed Value		
		Lamivudine	Dolutegravir	Tenofovir Alafenamide
1.	System suitability (mean)			
	Retention Time	3.142	4.612	6.792
	Theoretical Plate	5462	5762	6241
	c. Tailing Factor	0.621667	0.625	0.72
2.	Accuracy (% Recovery)	Mean Percent Recovery		
	80%	100.2	99.81	99.49
	100%	99.51	99.30	99.39
	120%	99.77	99.21	100.4
3.	Precision			
	Intraday precision (%RSD)	0.3	0.01	0.13
	Interday precision (%RSD)	0.31	0.01	0.12
4.	Linearity			
	Concentration range (mcg mL^{-1})	12-60	2-10	12-60
	Y=ax+b	y = 123.45x - 88.478	y = 100.14x - 201.99	y = 211.39x - 112.23
	R^2	0.9999	0.9994	0.9997
5.	LOD, Limit of Detection	0.12 µg/mL	0.21 µg/mL	0.24 µg/mL
6.	LOQ, Limit of Quantitation	0.36 µg/mL	0.34 µg/mL	0.73 µg/mL
7.	Robustness a. Flow rate (0.5 mL/min and 0.9 mL/min)	0.13, 0.46	0.06, 0.04	0.02, 0.30
	Mobile phase (Methanol: Buffer (65:35) and Methanol: Buffer (75: 25)	0.11, 0.03	0.13, 0.11	0.005, 0.02
	Wavelength (262 nm and 266 nm)	0.35, 0.05	0.001, 0.033	0.14, 0.05

Table 2: Assay results of Lamivudine, Dolutegravir and Tenofovir alafenamide.

Lamivudine				Dolutegravir			Tenofovir alafenamide		
Sl. No.	Standard Area	Sample area	% of Drug	Standard Area	Sample area	% of Drug	Standard Area	Sample area	% of Drug
1	1381	1385	100.28	305.03	305.02	99.99	1025.55	1025.51	99.99
2	1382	1386	100.28	305.04	305.01	99.99	1025.51	1025.53	100.0
3	1380	1384	100.28	305.01	305.04	100.0	1025.53	1025.52	99.99
4	1384	1385	100.07	305.02	305.03	100.0	1025.52	1025.51	99.99
5	1382	1386	100.28	305.03	305.01	99.99	1025.54	1025.53	99.99
6	1381	1383	100.14	305.04	305.02	99.99	1025.51	1025.54	100.0
Mean	1381	1383	100.22	305.02	305.03	99.99	1025.53	1025.51	99.99
Std dev	1.103	1.121	0.07	0.509	0.508	0.016	2.093	2.083	0.021
%RSD	0.08	0.074	0.075	0.166	0.161	0.017	0.206	0.208	0.175

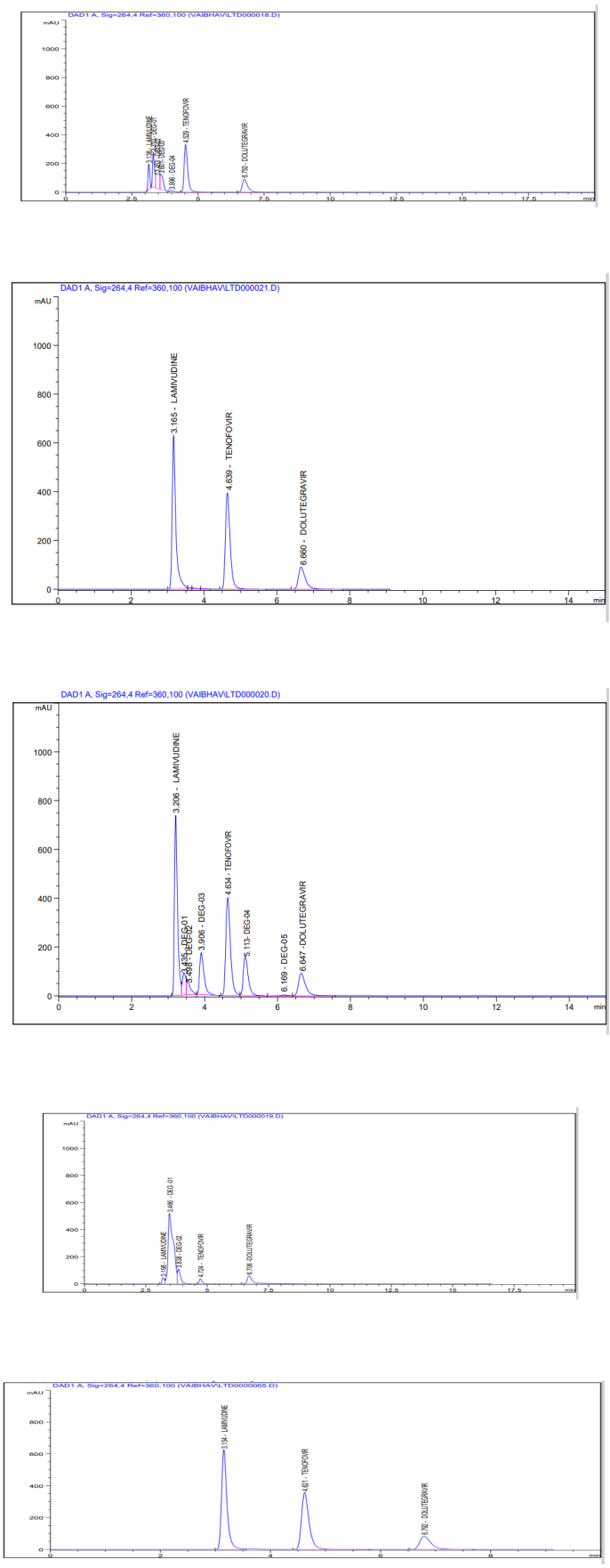
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Table 3: Degradation data of Lamivudine, Dolutegravir and Tenofovir alafenamide.

Drug substance	Degradation condition	%Drug degraded
Lamivudine	Acid	13.18
	Base	10.89
	Oxidation	1.65
	Thermal	1.69
	Neutral	2.29
Dolutegravir	Acid	0.51
	Base	10.85
	Oxidation	2.85
	Thermal	5.94
	Neutral	2.73
Tenofovir alafenamide	Acid	9.50
	Base	17.80
	Oxidation	17.85
	Thermal	2.93
	Neutral	0.30

IMAGE MISSING

Figure 2: (a-e)



storage. DGT was found to be relatively stable under most conditions, with base-induced degradation (10.85%) being the highest. Thermal degradation (5.94%), while oxidative (2.85%), neutral (2.73%), and acidic (0.51%) conditions caused minimal degradation. These results suggest that DGT is more prone to alkaline hydrolysis but stable under acidic and oxidative environments. TA exhibited significant degradation under oxidative (17.85%) and basic (17.80%) conditions, indicating high sensitivity to oxidation and alkaline media. Moderate degradation was observed under acidic (9.50%) and thermal (2.93%) conditions, while it remained stable under neutral conditions (0.30%). Hence, protection from oxidative and basic environments is crucial for the stability of TA formulations were calculated in Table 3. The findings demonstrated that the devised approach was adequate and that there was no interaction between excipients and drugs in the commercial formulation. The results indicate that the proposed approach is suitable for the routine assessment of these drugs' combination formulations.

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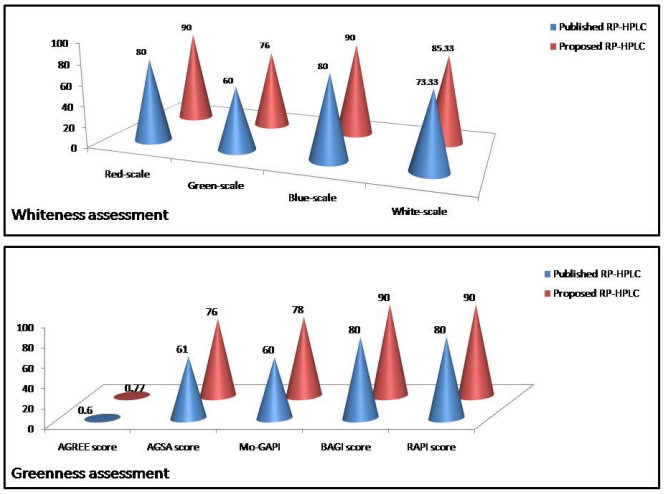


Figure 3: Greenness and whiteness profile assessment and comparison of published and proposed HPLC method.

Whiteness and greenness profile assessment

A comprehensive comparison of published and proposed RP-HPLC methods for the concurrent stability study of LMU, DTG and TA using RGB12 scales, Mo-GAPI, AGREE, BAGI and ASGA tools is presented in Table 4 and Figure 3. The assessment is divided into three main scales: red (validation efficiency), green (environmental friendliness) and blue (cost and time efficiency). The red scale evaluates aspects like scope, sensitivity, accuracy, and precision. The green scale considers factors such as toxicity, environmental impact, waste generation, and power consumption. The blue scale examines cost efficiency, time efficiency, analytical steps and user-friendliness. Finally, a white analytical scale combines these scores to provide an overall assessment of the methods' performance. This multi-faceted approach allows for a holistic comparison of the techniques, considering their analytical performance, environmental impact, and operational efficiency.[22]

Red-scales profile assessment

For the study of validation efficiency of published and proposed RP-HPLC methods, four principles for Red model-based assessment (R1 to R4; see Table 4) were applied as per ICH Q2 (R[1]) guidelines. The published RP-HPLC methods obtained a score of 80 out of 100 for all four principles for the estimation of LMU, TA and DTG. The proposed method has obtained 10 points extra for its scope and application due to the simultaneous estimation of LMU, TA and DTG. Hence, the proposed method has obtained a score of 90 out of 100 for the validation assessment by the Red model.[23,24]

Green-scale profile assessment

The green-scale analytical profile assessment compares the environmental friendliness of the published and proposed RP-HPLC methods for the concurrent stability study of LMU, DTG and TA. The proposed method demonstrates superior environmental performance across multiple parameters. It significantly reduces the use of toxic organic solvents, requiring less than 30 mL compared to over 100 mL in published methods. This reduction in solvent usage not only decreases the risks of human and animal toxicity but also minimizes environmental impact. The proposed method generates less than 100 mL of toxic organic waste, which is considerably lower than the published methods. However, power consumption remains similar, with both methods consuming more than 1.0 kwh. The environmental friendliness of the proposed method is further validated by higher scores in green analytical tools: AGREE (0.77 vs. 0.60), Mo-GAPI (78 vs. 60), and AGSA (76 vs. 61). Consequently, the proposed RP-HPLC method achieved a green-scale assessment score of 76 out of 100, significantly outperforming the published methods' score of 60, thus demonstrating its superior environmental efficiency for the concurrent stability study of LMU, DTG, and TA.[25]

Blue-scale profile assessment

The blue-scale analytical profile assessment compares the cost and time efficiency of the published and proposed RP-HPLC methods for the concurrent stability study of LMU, DTG and TA. The proposed method demonstrates superior performance across multiple parameters. It is more cost-efficient, requiring fewer resources for concurrent stability study and bioanalysis compared to the published methods. The proposed method is also more time-efficient, as it requires less time for analysis. In terms of analytical steps, the proposed method is simpler and easier to handle, requiring fewer steps for the concurrent stability study. The proposed method is more user-friendly, as it utilizes a single chromatographic condition instead of the multiple conditions required by published methods. These improvements in efficiency and usability contribute to the proposed RP-HPLC method achieving a blue-scale assessment score of 90 out of 100, outperforming the published methods' score of 80. This higher score indicates that the proposed method is more cost-effective, time-efficient, and user-friendly for the concurrent stability study of LMU, DTG, and TA.[26]

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Table 4: White analytical chemistry profile assessment of proposed and published RP-HPLC methods for concurrent stability study of Lamivudine, Dolutegravir, and Tenofovir using RGB12 scales, Mo-GAPI, AGREE, BAGI, and ASGA tools.

RGB12 multi-colored analytical chemistry scale	Reported RP-HPLC methods for concurrent stability study of Lamivudine, Dolutegravir and, Tenofovir alafenamide	Proposed RP-HPLC method for concurrent stability study of Lamivudine, Dolutegravir and, Tenofovir alafenamide
Red analytical scale for assessment of validation efficiency		
R^1 -scopes and applicability	No stability-indicating RP-HPLC method has been reported in the literature to date for the concurrent stability-indicating estimation of LMU, DTG, and TA.	This study develops and validates a RP-HPLC method for the simultaneous estimation of LMU, DTG, and TA in pharmaceuticals. It's a "unique, simple, successful, quick and accurate stability indicating" technique, compliant with ICH requirements. Applications include quality control, stability testing, forced degradation studies and regulatory compliance in the analysis of HIV medications.
R^2 -Sensitivity (LOD and LOQ)	The reported individual RP-HPLC methods for stability studies of LMU, DTG, and TA exhibit sensitivity at the microgram level using separate and dedicated chromatographic conditions.	The RP-HPLC method's sensitivity is demonstrated by its Limit of Detection (LOD) and Limit of Quantification (LOQ) values. For LMU, DTG, and TA, the LOD values are 0.12, 0.11 and 0.24 µg/mL, while the LOQ values are 0.36, 0.34, and 0.73 µg/mL, respectively. These low values indicate high sensitivity, crucial for accurate pharmaceutical analysis and quality control.
R3 -Accuracy	The reported individual RP-HPLC method for stability study of LMU, DTG, and TA complied with the accuracy study according to ICH guidelines. However, it required more resources for the validation study.	The RP-HPLC method's accuracy was rigorously evaluated through recovery studies conducted at three distinct concentration levels: 80%, 100%, and 120%. The results demonstrated consistently high recovery percentages for all three drugs under analysis. LMU showed recovery rates of 100.2%, 99.51%, and 99.77% at the respective concentration levels. DTG exhibited recovery percentages of 99.81%, 99.30%, and 99.21%, while TA displayed 99.49%, 99.39% and 100.4% recovery.
R4 -Precisions	The reported individual RP-HPLC method for stability study of LMU, DTG and TA complied for precision study with ICH guidelines. But required more resources for validation study.	The precision study evaluated intraday and interday variability for LMU, DTG, and TA. Results showed low %RSD values: LMU (intraday: 0.3%, interday: 0.31%), DTG (intraday: 0.01%, interday: 0.01%), and TA (intraday: 0.13%, interday: 0.12%) . These low %RSD values indicate high precision and reproducibility of the RP-HPLC method for all three drugs.
Red scale assessment score	Hence, the published RP-HPLC methods have obtained red-scale assessment score of 80 out of 100 for concurrent stability study of LMU, DTG and TA.	Hence, the proposed RP-HPLC method has obtained red-scale assessment score of 90 out of 100 for concurrent stability study of LMU, DTG and TA.
Green analytical scale for assessment of environment friendliness using AGSA, Mo-GAPI and AGREE tools		
G1 -Human and animal toxicity	The individual published RP-HPLC method for concurrent stability –indicating estimation of LMU, DTG and TA required more than 100 mL of toxic organic solvents such as methanol and acetonitrile.	The proposed RP-HPLC method for concurrent stability –indicating estimation of LMU, DTG and TA required less than 30 mL of toxic organic solvents such as methanol.

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Table 4: (Continued)

RGB12 multi-colored analytical chemistry scale	Reported RP-HPLC methods for concurrent stability study of Lamivudine, Dolutegravir and, Tenofovir alafenamide	Proposed RP-HPLC method for concurrent stability study of Lamivudine, Dolutegravir and, Tenofovir alafenamide
G2 -Environmental impacts	The acetonitrile and methanol used in reported individual RP-HPLC method for concurrent stability study of LMU, DTG and TA are hazardous to the environment.	In the proposed RP-HPLC method eco-friendly mobile phase used, Methanol and TEA buffer for stability study of LMU, DTG and TA used in is less hazardous to the environment than published methods.
G3 -Toxic waste generation	The individual published RP-HPLC method for concurrent stability study of LMU, DTG and TA have generated more than 100 mL toxic organic waste.	The proposed RP-HPLC method for concurrent stability study of LMU, DTG and TA is generating less than 100 mL toxic organic waste. It is always less than published methods
G4 -Power consumption	The power consumption of the published RP-HPLC methods is more than 1.0 KWh.	The power consumption of the proposed RP-HPLC method is more than 1.0 KWh.
Green analytical tools	The AGREE tool showed score of 0.60 out of 1.0 for published RP-HPLC method. Mo-GAPI tool showed score of 60 out of 100 for published RP-HPLC method. AGSA tool showed score of 61 out of 100 for published RP-HPLC method.	The AGREE tool showed score of 0.77 out of 1.0 for proposed RP-HPLC method. Mo-GAPI tool showed score of 78 out of 100 for proposed RP-HPLC method. AGSA tool showed score of 76 out of 100 for proposed RP-HPLC method.
Green scale assessment scores	Hence, the published RP-HPLC methods have obtained green-scale assessment score of 60 out of 100 for concurrent stability study of STM and GMP.	Hence, the proposed RP-HPLC method has obtained green-scale assessment score of 76 out of 100 for concurrent stability study of STM and GMP.
Blue analytical scale for assessment of cost and time efficiency using BAGI tool		
B1 -Cost efficiency	The published individual RP-HPLC methods have required more resources for concurrent stability study and bioanalysis of LMU, DTG and TA. Hence, the published RP-HPLC methods are more costly than proposed RP-HPLC method.	The proposed RP-HPLC methods have required fewer resources for concurrent stability study and bioanalysis of LMU, DTG and TA in human plasma. Hence, the proposed RP-HPLC method is more economical than published RP-HPLC methods.
B2 -Time efficiency	The published individual RP-HPLC methods have required more time for concurrent stability study and bioanalysis of LMU, DTG and TA. Hence, the published RP-HPLC methods are more time consuming than proposed RP-HPLC method.	The proposed RP-HPLC methods have required less time for concurrent stability study and bioanalysis of LMU, DTG and TA in human plasma. Hence, the proposed RP-HPLC method is faster than published RP-HPLC methods.
B3 -Analytical steps	The published individual RP-HPLC methods have required more analysis steps for concurrent stability study of LMU, DTG and TA. Hence, the published RP-HPLC methods are more complex than proposed RP-HPLC method.	The proposed RP-HPLC method has required less analytical steps for concurrent stability study of LMU, DTG and TA in human plasma. Hence, the proposed RP-HPLC method is easier to handle than published RP-HPLC methods.

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Table 4: (Continued)

RGB12 multi-colored analytical chemistry scale	Reported RP-HPLC methods for concurrent stability study of Lamivudine, Dolutegravir and, Tenofovir alafenamide	Proposed RP-HPLC method for concurrent stability study of Lamivudine, Dolutegravir and, Tenofovir alafenamide
B4 -User friendliness	The proposed RP-HPLC methods for concurrent stability study of LMU, DTG and TA is less user-friendly as it required multiple chromatographic conditions to set. Hence, the proposed RP-HPLC methods are less user-friendly than published RP-HPLC methods.	The proposed RP-HPLC method for concurrent stability study of LMU, DTG and TA is more user-friendly as it required single chromatographic condition to set. Hence, the proposed RP-HPLC method is more user-friendly than published RP-HPLC methods.
Blue scale assessment score	Hence, the published RP-HPLC methods have obtained blue-scale assessment score of 80 out of 100 for concurrent stability study of LMU, DTG and TA	Hence, the published RP-HPLC methods have obtained blue-scale assessment score of 90 out of 100 for concurrent stability study of LMU, DTG and TA
White analytical scale for assessment of multicoloured analytical efficiency using RGB12 scales		
Whiteness profile scored	The whiteness profile score of the published RP-HPLC methods have obtained score of 73.33 average of (80+ 60+80).	The whiteness profile score of the proposed RP-HPLC method has obtained score of 85.33 average of (90+76+ 90).
Whiteness status	Hence, the published individual RP-HPLC methods using multiple chromatographic conditions are enough white (whiteness profile score of less than 75) for concurrent stability study of LMU, DTG and TA	Hence, the proposed RP-HPLC methods using single chromatographic conditions is excellent white (whiteness profile score of more than 85) for concurrent stability study of LMU, DTG and TA

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White-scale profile assessment

The white-scale analytical profile assessment provides a comprehensive evaluation of the published and proposed RP-HPLC methods for concurrent stability study of LMU, DTG, and TA, integrating the red, green, and blue scale assessments. The proposed method demonstrates superior overall performance compared to the published methods. The whiteness profile score, which is an average of the red, green, and blue scale scores, is significantly higher for the proposed method at 85.33 (90 + 76 + 90)/3, compared to 73.33 (80 + 60 + 80)/3 for the published methods. This higher score reflects the proposed method's improvements in analytical efficiency, environmental friendliness, and cost-time efficiency. Notably, the proposed method achieves an 'excellent white' status with a whiteness profile score exceeding 85, while the published methods are classified as 'enough white' with a score below 75. A key factor contributing to this superior performance is the proposed method's use of a single chromatographic condition, as opposed to the multiple conditions required by the published methods. This comprehensive assessment underscores the overall superiority of the proposed RP-HPLC method for the concurrent stability study of LMU, DTG, and TA, demonstrating its potential to enhance analytical processes in pharmaceutical research and quality control.[27] Green chemistry principles advocate reducing hazardous chemicals, minimizing waste, and enhancing energy efficiency. In HPLC analysis, solvent consumption is a significant environmental concern; thus, greenness assessment tools are applied to quantify and reduce the ecological impact. systematically evaluating and optimizing critical method parameters, enhancing robustness and sensitivity beyond previously reported.[28,29]

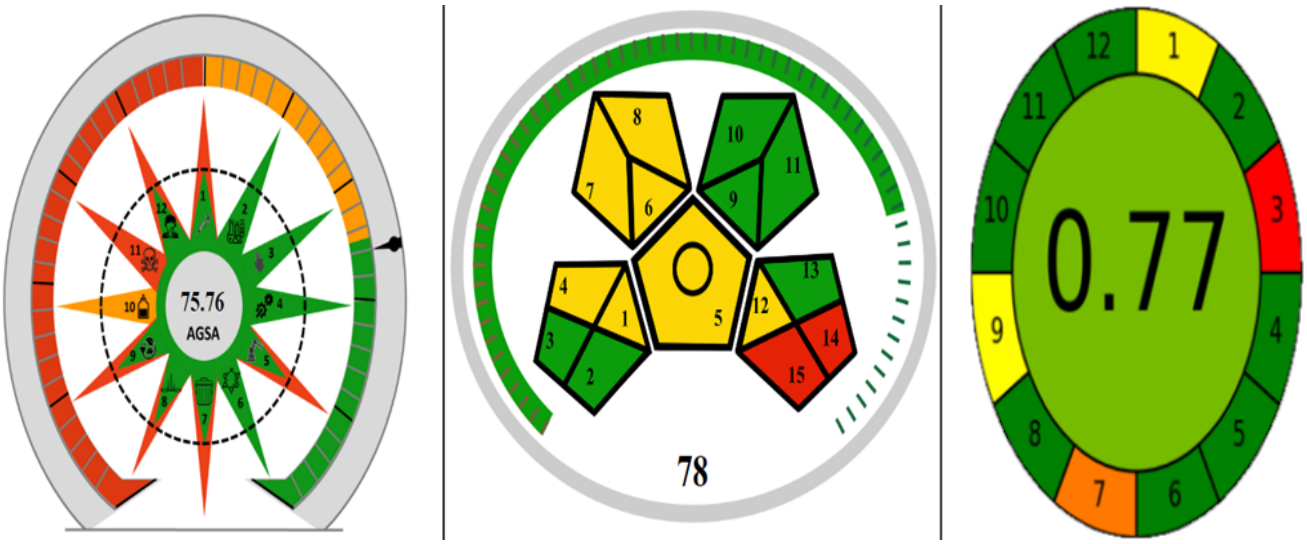


Figure 4: Representing white analytical metric using with using ecofriendly tools AGREE (0.77), Mo-GAPI (78), and AGSA (76).

CONCLUSION

This study developed and validated a simple, reliable, and stability-indicating reverse-phase HPLC method for the simultaneous estimation of LMU, DTG, and TA in pharmaceutical formulations. The method demonstrated excellent linearity (R^2> 0.9999, 0.9997, and 0.9994), recoveries of LMU, DTG and TA for 80% was found to be 100.2%, 99.81% and 99.49 respectively, for 100% was found to be 99.51%, 99.30% and 99.39% and for 120% was found to be 99.77%, 99.21% and 100.4% respectively and precision (intraday %RSD: 0.3, 0.01, 0.13; interday %RSD: 0.31, 0.01, 0.12) within limit. Sensitivity was confirmed with low limits of detection (0.12, 0.24, 0.21 µg/mL) and quantification (0.36, 0.73, 0.34 µg/mL). The method complied with ICH Q2 (R^2) guidelines. Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and hydrolytic conditions confirmed the method's stability-indicating capability. Degradation products were well separated and did not interfere with drug peaks, establishing specificity. Environmental impact was minimized through green analytical approaches, with favorable scores from AGREE (0.77), Mo-GAPI (78), and AGSA (76). The RGB model further supported sustainability, yielding a high overall score (85.33), indicating eco-friendly performance. The method is also cost-effective compared to conventional chromatographic techniques. Overall, the proposed isocratic RP-HPLC method is simple, precise, and suitable for routine analysis of LMU, DTG, and TA in pharmaceutical laboratories. All three compounds were successfully separated using the recommended strategy, and the active contents were also calculated. The suggested plan is stability-indicating and specific. It ensures accurate quantification, effective separation under stress conditions, and supports both regulatory compliance and environmentally conscious practices

SUMMARY

The simultaneous measurement of LMU, DTG, and TA in tablet formulation was accomplished through the development and validation of a novel, simple, and environmentally friendly RP-HPLC method. Under ideal chromatographic conditions, the approach demonstrated good linearity (R^2> 0.999), accuracy (≈99%), precision (%RSD < 0.5), and sensitivity, with a distinct separation. It confirmed specificity by successfully indicating stability under a range of stress settings free from interference from degradation products. With a high whiteness score (85.33), environmental assessment techniques (AGREE=0.77, Mo-GAPI=78, AGSA=76) showed its exceptional sustainability and greenness. Thus, the method is accurate, robust, cost-effective, and environmentally sustainable for routine quality control of LMU, DTG, and TA in pharmaceuticals.

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ABBREVIATIONS

LMU: Lamivudine; **DTG**: Dolutegravir; **TA:** Tenofovir Alafenamide; **RP-HPLC:** Reverse phase High-performance liquid chromatography; **LOD:** Limit of detection; **LOQ:** Limit of Quantitation; **ICH:** International Conference on harmonization; **HIV:** Human Immunodeficiency Virus; **NRTI:** nucleoside reverse transcriptase inhibitor; **WAC:** White Analytical Chemistry; **GAC:** Green Analytical Chemistry; **API:** Active pharmaceutical ingredients; **SD:** Standard deviation; **EHS:** Environmental, health and safety; **AGREE:** Analytical GREEness Calculator; **Mo-GAPI:**

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Modified Green Analytical Procedure Index; **AGSA:** Analytical Green Star Area; **OPA:** Ortho-Phosphoric Acid; **T**: Tailing factor; **N:** Theoretical plate number; **Rs:** Resolution; **Rt:** Retention time; **UV:** Ultraviolet; **HPTLC:** High-Performance Thin-Layer Chromatography; **R^2:** Regression coefficient; **%RSD:** Relative standard deviation; **H_2O_2:** Hydrogen peroxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS CONTRIBUTION

Vaibhav Sawale: Conceptualization, Investigation, Writing-Original Draft.

Roshni Kunte: Data acquisition, Writing- original draft, Prafulla.

Sabale: Supervision and Reviewing the manuscript.

Vidya Sabale: Validation and Visualization.

REFERENCES

1. Garvey EP, Johns BA, Gartland MJ, Foster SA, Miller WH, Ferris RG., *et al.*; The naphthyridinone GSK364735 is a novel, potent human immunodeficiency virus type 1 integrase inhibitor and antiretroviral; Antimicrobial Agents and Chemotherapy. 2008; 52-3: 901-908. https://doi.org/10.1128/aac.01218-07
2. Corado KC, Caplan MR, Daar ES. Two-drug regimens for treatment of naive HIV-1 infection and as maintenance therapy. Drug Design Development and Therapy. 2018; 12-7:3731-3740. https://doi.org/10.2147/DDDT.S140767.
3. Kandel CE, Walmsley SL. Dolutegravir–a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Design Development and Therapy. 2015; 3-7:3547-3555. https://doi.org/10.2147/DDDT.S84850
4. Maggiolo F, Gulminetti R, Pagnucco L, Digaetano M, Benatti S, Valenti D, Callegaro A, *et al.*; Lamivudine/dolutegravir dual therapy in HIV-infected, virologically suppressed patients. BMC Infectious Diseases. 2017;17-1:215.https://doi.org/10.1186/s12879-017-2311-2
5. Mastanamma SK, Jyothi JA, Saidulu P, Varalakshmi M. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir Bulk and their Combined Dosage Form. Pharmaceutical Methods. 2018;9-2;49-55. https://doi.org/10.5530/phm.2018.2.10
6. Deepali G, Elvis M. UV Spectrophotometric method for assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredient in its tablet formulation. Journal of Young Pharmacists. 2010; 2-4:417-419. https://doi.org/10.4103/0975-1483.71628
7. Avhad S, Morkar V, Shinde S, Patki C, Chikhale H, Borse L. Recent advances in analytical method development and validation techniques for anti-HIV pharmaceuticals of tenofovir. Biosciences Biotechnology Research Asia. 2023;20-2:407-432. http://dx.doi.org/10.13005/bbra/3099
8. Bhavar GB, Pekamwar SS, Aher KB, Thorat RS, Chaudhari SR. High-performance liquid chromatographic and high-performance thin-layer chromatographic method for the quantitative estimation of dolutegravir sodium in bulk drug and pharmaceutical dosage form. Scientia Pharmaceutica. 2015;84-2:305. http://doi.10.3797/scipharm1507-09.
9. Kumar GR, Rao RR, Vardhan VM, Abbaraju VK. An Effective Stability Indicating RP-HPLC Method for Simultaneous Estimation of Lamivudine and Raltegravir in Bulk and Their Tablet Dosage Form. Journal of Pharmaceutical Research International. 2021;33-45B:263-277. http://10.9734/jpri/2021/v33i45b32803.
10. M. A. Pathan and A. Kshirsagar, "Stability Indicating HPLC Method Development and Validation for Simultaneous Estimation of Metformin and Empagliflozin in Bulk and Pharmaceutical Dosage Form," Research Journal of Pharmacy and Technology, 2022;15-2:830–836. http://10.52711/0974-360X.2022.00138.
11. Haggag RS, Belal SF, Hewala II. Stability-Indicating HPLC-DAD Determination of Lamivudine in Pharmaceutical Preparations and Biological Fluids. Current trends in Analytical and Bioanalytical Chemistry. 2021;5-1:150-7. 10.25258/ijpqa.15.4.85
12. Singh AV, Nath LK, Pani NR. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. Journal of Pharmaceutical Analysis. 2011-1-4:251-257. https://doi.org/10.1016/j.jpha.2011.08.001
13. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J. White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality. Trends in Analytical Chemistry. 2021;1-138:116-223. https://doi.org/10.1016/j.trac.2021.116223
14. Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. Trends in Analytical Chemistry. 2013 Oct 1;50:78-84. https://doi.org/10.1016/j.trac.2013.04.010
15. Levent S, Elriş A, Avcı H, Özcan S, Can NÖ. Chemometrically assisted optimization and validation of the HPLC method for the analysis of alectinib and its related impurity with measurement uncertainty evaluation and whiteness, blueness, and greenness profile assessments. BMC Chemistry. 2025;19-1:147-157. https://doi.org/10.1186/s13065-025-01509-y
16. Mahgoub SM, Alminderej FM, Binsaleh AY, Alsehli BR, Saleh SM, Mohamed MA. Green and white analytical approach for parallel quantification of gabapentin and methyl cobalamin in medicinal products using inventive RP-HPLC technique. Scientific Reports. 2025;15-1:202-263. https://doi.org/10.1038/s41598-025-07056-9
17. Prajapati PB, Jayswal K, Shah SA. Application of quality risk assessment and DoE-based enhanced analytical quality by design approach to development of chromatography method for estimation of combined pharmaceutical dosage form of five drugs. Journal of Chromatographic Science 2021 59-8: 714-729. https://doi.org/10.1093/chromsci/bmaa118
18. Boggavarapu A, Vegesna S. A Review on Analytical Methods for Determination of Pioglitazone with RBG and White Analysis. Journal of Pharma Insights and Research. 2025;3-3:117-126. https://doi.org/10.69613/ba35sx50
19. Prajapati P, Shahi A, Acharya A, Pulusu VS, Shah S. Implementation of white analytical chemistry–assisted analytical quality by design approach to green liquid chromatographic method for concomitant analysis of anti-hypertensive drugs in human plasma. Journal of Chromatographic Science. 2024;62-10:938-952. https://doi.org/10.1093/chromsci/bmad054
20. Harmonised Tripartite Guideline ICH (2023) Validation of Analytical Procedures: Text and Methodology Q2(R^2) Current Step 4 Version.
21. Harmonised Tripartite Guideline ICH (2003) Stability Testing of New Drug substances and Products Q1A(R^2) Current Step 4 version.
22. Prajapati P, Patel H, Shah S. Quality risk assessment and DoE-based analytical quality by design approach to stability-indicating assay method for acidic degradation kinetic study of apremilast. Journal of Planar Chromatography–Modern TLC. 2020;33-3:231-244. https://doi.org/10.1007/s00764-020-00036-x
23. Prajapati PB, Radadiya K, Shah SA. Quality risk management based: analytical quality by design approach to eco-friendly and versatile chromatography method for simultaneous estimation of multiple fixed-dose-combination products of anti-diabetic drugs. Journal of Pharmaceutical Innovation. 2022;17-1:111-128. https://doi.org/10.1007/s12247-020-09506-5
24. Abdelgawad MA, Abdelaleem EA, Gamal M, Abourehab MA, Abdelhamid NS. A new green approach for the reduction of consumed solvents and simultaneous quality control analysis of several pharmaceuticals using a fast and economic RP-HPLC method; a case study for a mixture of piracetam, ketoprofen and omeprazole drugs. Royal Society of Chemistry advances. 2022;12-25:16301-16309. DOI: 10.1039/D2RA02395D
25. Prajapati PB, Sheta BM, Pulusu V, Shah SA. Analytical quality risk assessment and design of experiments to green HPTLC method for simultaneous estimation of sildenafil citrate and dapoxetine hydrochloride. Journal of Chromatographic Science. 2024;62-5:454-464. https://doi.org/10.1093/chromsci/bmad057
26. Hussain CM, Hussain CG, Keçili R. White analytical chemistry approaches for analytical and bioanalytical techniques: Applications and challenges. Trends in Analytical Chemistry. 2023;1-59:116-905. https://doi.org/10.1016/j.trac.2022.116905
27. Prajapati P, Pulusu VS, Shah S. White analytical chemistry-driven stability-indicating concomitant chromatographic estimation of thiocolchicoside and aceclofenac using response surface analysis and red, green, and blue model. Journal of Separation Science. 2023;46-11:118-125. https://doi.org/10.1002/jssc.202300139
28. Ashok H. Akabari, Ketan V. Shah, Jayesh Kushwaha, Jasmina Surati, Ghanshyam G. Tejani, Sarfaraz Ahmed and Seyed Jalaleddin Mousavirad (2026) White analytical chemistry–aligned eco-friendly, stability-indicating HPLC and HPTLC methods for simultaneous estimation of efonidipine hydrochloride ethanolate and chlorthalidone, Journal of Taibah University for Science, 20:1, 2606450, DOI: 10.1080/16583655.2025.2606450
29. Rabadiya, V. A., Shah, N., and Akabari, A. H. (2025). Eco-friendly RP-HPLC method for simultaneous estimation of amlodipine besylate and indapamide: analytical quality by design approach and greenness assessment. *Separation Science Plus*, *8*(3), https://doi.org/10.1002/sscp.70019

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