

AI-assisted design and optimization of two smart green HPLC methods for simultaneous determination of some antiviral drugs

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ABSTRACT

Two novel high-performance liquid chromatography (HPLC) methods were developed and validated for the determination of dolutegravir (DTG), lamivudine (3TC), and abacavir (ABA) in pharmaceutical formulations. In this study, AI engines such as Copilot, ChatGPT 5.2, Gemini, and Perplexity were integrated into HPLC method development. The selected predicted conditions **underwent** experimental optimization, validation, and refinement. Their practical implementation significantly improved efficiency and accuracy in the analytical process. The first method, an isocratic HPLC, was designed for the simultaneous quantification of DTG and 3TC using an XBridge® C18 column and a mobile phase of acetonitrile:phosphate buffer (pH 3.5, 50:50 v/v). Detection was performed at 258.0 nm for DTG and 275.0 nm for 3TC. The second method, a gradient HPLC, enabled the simultaneous quantification of DTG, 3TC, and ABA on a Spherisorb® ODS2 C18 column with methanol and TEA/TFA buffer (pH 3.15) as the mobile phase. Detection wavelengths were 258.0, 278.0, and 294.0 nm for DTG, 3TC, and ABA, respectively. The calibration ranges were wide and showed excellent linearity. Both techniques were validated in accordance with ICH Q2(R2) guidelines. The approaches align with smart analytical chemistry principles, combining green and white analytical chemistry (GAC and WAC) with AI-driven method development, resulting in accurate, fast, and sustainable techniques suitable for routine quality control applications.

1. Introduction

The newest family of antiretrovirals, well-tolerated integrase strand transfer inhibitors (INSTI) show strong anti-HIV effectiveness by blocking the enzyme that integrates viral DNA into the host genome [1]. Dolutegravir (DTG), a novel INSTI, is a chiral, non-racemic substance. Its chemical name is (4R,9aS)-5-hydroxy-4-methyl-6,10-dioxo-3,4,6,9,9a,10-hexahydro-2H-1-oxa-4a,8a-diaza-anthracene-7-carboxylic acid-2,4-difluorobenzylamide (Fig. 1a). While conformational alterations in the pocket shape occur, DTG can bind by fitting loosely into the intake binding pocket. Because of its heightened genetic resistance to antiretroviral resistance and its capacity to alter its binding position, dolutegravir is classified as a second-generation INSTI [2,3].

Lamivudine (3TC) is an antiretroviral drug used to prevent and treat AIDS (Fig. 1b). 3TC is used with other drugs, including abacavir (ABA), DTG, and zidovudine. The Chemical structure of 3TC is 4-amino-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl) pyrimidin-2-one [4]. ABA is, [(1S,4R)-4-[2-amino-6-(cyclopropylamino) purin-9-yl] cyclopent-2-en-1-yl] methanol, substance used to treat AIDS (Fig. 1c) [5].

Several studies have reported the simultaneous quantification of these active substances, including investigations using HPLC with analysis times of 11.0, 13.0, and 17.0 min [6–8]. In contrast, the proposed method completes the analysis in just 6.0 min. The shorter analysis time enables a more environmentally friendly procedure through reduced energy consumption, less waste generation, and faster

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throughput. Additionally, the developed method allows quantification at lower concentrations, demonstrating its high precision. In previous studies, the lowest concentrations reported for 3TC and ABA were 15.0 and 30.0 $\mu\text{g/mL}$, respectively [6,7], whereas in the present study, these levels were reduced to 5.0 $\mu\text{g/mL}$ for 3TC and 10.0 $\mu\text{g/mL}$ for ABA. As an alternative approach, determination of all three active substances has been reported using chemometric methods [9]. However, these methods require specialized software for analysis.

Given the clinical significance of combination therapy in the management of HIV infection, simultaneous analytical techniques capable of quantifying individual and multiple active pharmaceutical ingredients (APIs) such as dolutegravir (DTG), lamivudine (3TC), and abacavir (ABA) are more important. A comprehensive review indicates that there is no available HPLC method that provides rapid analysis suitable for routine quality control. Furthermore, current publications do not address method evaluation from a green chemistry perspective, which is increasingly vital given the global emphasis on sustainable analytical practices and environmental protection.

Integration of AI in the HPLC method development is not only cost-effective but also aligned with green chemistry principles, making them highly applicable for routine quality control testing while contributing to environmentally sustainable analytical practices [10].

Under the lens of Green Chemistry and the broader sustainability paradigm, a new concept known as Smart Analytical Chemistry has emerged [11]. This modern approach to method development encompasses three interconnected criteria that ensure analytical methods are not only effective but also environmentally responsible, socially relevant, and technologically advanced.

The first pillar of smart analytical chemistry is the alignment with GAC principles [12], which emphasize minimizing environmental impact by reducing solvent use, energy consumption, and waste generation. In this work, we applied a greenness evaluation tool named AGREE.

The second criterion is the integration of WAC [13], which expands the evaluation beyond environmental aspects to include analytical performance and practical applicability in real-world settings. For this purpose, we utilized the RGB12 algorithm, a recently proposed comprehensive tool that quantitatively evaluates an analytical method's balance of green, red (analytical efficiency), and blue (applicability) attributes.

The third component reflects the growing role of AI in the design and optimization of analytical methods. AI enables data-driven modeling, pattern recognition, and predictive capabilities, facilitating faster and more precise method development with reduced trial-and-error experimentation.

AI provides powerful tools that can greatly streamline the design of analytical procedures, optimize experimental conditions, and minimize the use of reagents and solvents. However, it is crucial to recognize that AI does not replace the expertise of the analytical chemist. Instead, it functions as an intelligent assistant, generating suggestions that require critical evaluation, guidance, and refinement by experienced professionals. Implementing AI-recommended methods involves careful adjustments and comprehensive validation to confirm their reliability, accuracy, and adherence to established analytical standards. Consequently, while AI tools can propose similar or alternative approaches, their integration into analytical workflows must always be supported by thorough validation processes. This ensures that AI remains a valuable complementary asset, enhancing, rather than replacing, the expertise necessary for developing robust and efficient analytical solutions.

Aligned with this intelligent analytical framework, various artificial intelligence engines such as Copilot, ChatGPT 5.2, Gemini and Perplexity were integrated into the HPLC analysis. The proposed AI-driven methodologies were subjected to experimental optimization, validation and iterative refinement. Through practical implementation of AI-generated suggestions, they were optimized to enhance both efficiency and accuracy. Thus, simple, fast, environmentally benign HPLC techniques were optimized and validated for the simultaneous quantification of DTG, 3TC, and ABA in pharmaceutical preparations. The isocratic HPLC method for the determination of DTG and 3TC, and a gradient HPLC method for the simultaneous quantification of DTG, 3TC, and ABA in lab-prepared mixtures and their formulation, were systematically evaluated. Both methods align with the principles of GAC, WAC, and AI-enhanced optimization, making them model examples of smart, sustainable, and efficient analytical strategies.

2. Experimental

2.1. Instrument and software

HPLC experiments were conducted using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Photodiode Array Detector (PDA). Specimen preparation was carried out using an Elmasonic S30H ultrasonic bath sonicator (Elma Schmidbauer GmbH, Singen, Germany). Ultra-pure water was obtained using an ELGA PureLab purification system (ELGA LabWater, High Wycombe, UK), and pH measurements were performed with a Jenway 3510 digital pH meter (Jenway, Staffordshire, UK).

Chromatographic separations were achieved using two different reversed-phase columns according to the specificity and performance:

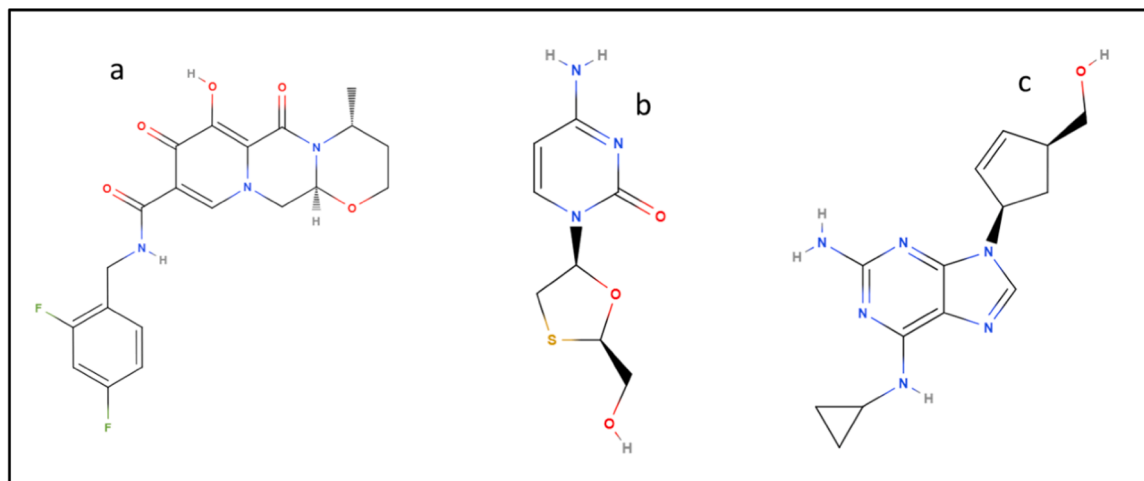


Fig. 1. Chemical structure of dolutegravir (DTG) (a), lamivudine (3TC) (b), and abacavir (ABA) (c).

- **For Isocratic Method: XBridge® C18 column** (5 μm , 4.6 \times 250 mm; Waters Corporation, Milford, MA, USA)
- **For Gradient Method: Spherisorb® ODS2 C18 column** (3.5 μm , 4.6 \times 100 mm; Waters Corporation, Milford, MA, USA)

Software: Microsoft Copilot (Microsoft Corporation, Redmond, WA, USA) was used to support optimization of chromatographic conditions.

2.2. Materials

2.2.1. Authentic specimens, chemicals, and solvents

- Dolutegravir (DTG), Abacavir (ABA), and Lamivudine (3TC) reference standards were obtained from the United States Pharmacopeia (USP, Rockville, MD, USA). The purity of each standard was confirmed using a previously reported method [9], yielding the following results: DTG: $99.92 \pm 0.22\%$, 3TC: $99.88 \pm 0.32\%$, and ABA: $100.02 \pm 0.31\%$.
- Sodium dihydrogen phosphate, triethylamine (TEA), trifluoroacetic acid (TFA), phosphoric acid, and acetonitrile were purchased from Merck (Darmstadt, Germany).

2.2.2. Excipients

Excipients used in the formulation studies: mannitol, microcrystalline cellulose, povidone, sodium starch glycolate, sodium stearyl fumarate, magnesium stearate, macrogol, hypromellose, and titanium dioxide—were also obtained from Merck (Darmstadt, Germany).

All mobile phases and solvents were freshly prepared and filtered through a 0.22 μm polyethersulfone (PES) membrane filter (MilliporeSigma, Burlington, MA, USA) before use to remove particulate matter and ensure chromatographic integrity.

2.2.3. Formulations

- *Triumeq®* film-coated tablets contain a fixed-dose combination of 50 mg DTG, 300 mg 3TC, and 600 mg ABA, produced by GlaxoSmithKline Industries and sourced from a local pharmacy.
- *In-house formulation* containing 50 mg DTG and 300 mg 3TC was prepared in the laboratory by blending the active pharmaceutical ingredients with appropriate excipients mentioned in Section 2.2.2.

3. Procedure

3.1. Chromatographic conditions

Two HPLC techniques were developed: an isocratic method for binary mixtures of 3TC and DTG, and a gradient technique for the simultaneous quantification of 3TC, DTG, and ABA in a ternary mixture. Initially, chromatographic parameters were estimated using the Copilot-AI modeling tool to predict suitable analytical conditions. Then, through laboratory experiments, these AI-generated settings were improved and perfected.

3.1.1. Isocratic method

Analysis was conducted using a Waters XBridge® C18 column (5 μm , 4.6 \times 250 mm). The mobile phase consisted of phosphate buffer (pH 3.5) and acetonitrile in a 50:50 (v/v) ratio. The injection volume was 10 μL , the column temperature was kept at 35 $^{\circ}\text{C}$, and the flow rate was chosen at 1.2 mL/min. Detection was performed using a PDA detector at 258.0 nm for DTG and 275.0 nm for 3TC. The total run time was <5 min.

3.1.2. Preparation of mobile phase (Isocratic method)

Phosphate buffer (0.02 M, pH 3.5) was prepared by dissolving 3.12 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in 800 mL of Type I water. Then, 2 mL of TEA was added, the pH was set to 3.5 ± 0.01 with (1 M) H_3PO_4 , and the solution volume was made up to 1 L. The buffer was mixed with ACN (50:50 v/v),

and the resulting solution was filtered through a 0.22 μm PES membrane filter.

3.1.3. Gradient method

Separation was conducted on a Waters Spherisorb® ODS2 C18 column (3.5 μm , 4.6 \times 100 mm). The mobile phases used were:

- Mobile Phase A: 0.175 % TEA + 0.1 % TFA in water (pH adjusted to 3.15)
- Mobile Phase B: Methanol (HPLC-grade).

The gradient elution was as follows:

0.0–2.0 min: A/B = 50:50 v/v

2.1–4.0 min: A/B = 0:100 v/v

4.2–6.0 min: return to A/B = 50:50 v/v

Flow rate was 1.2 mL/min, the column temperature was adjusted at 40 $^{\circ}\text{C}$, and the injection volume was 5 μL . The PDA detector was set to 258.0 nm for DTG, 278.0 nm for 3TC, and 294.0 nm for ABA. All mobile phases were degassed using an ultrasonic bath at 20 $^{\circ}\text{C}$.

3.1.4. Preparation of mobile phase (Gradient method)

Mobile Phase A was prepared by adding 3.5 mL of TEA and 2.0 mL of TFA to 2.0 L of Type I water. The solution was capped, shaken to ensure homogeneity, and filtered through a 0.22 μm PES membrane filter. Mobile Phase B consisted of methanol was also filtered through a 0.22 μm PES membrane filter.

3.1.5. Preparation of standard solutions

Stock solutions (500 $\mu\text{g}/\text{mL}$) of DTG, ABA, and 3TC were prepared in 50 mL of mobile phase and stored at 4 $^{\circ}\text{C}$. Calibration standard solutions were made by serial dilution, filtered through 0.22 μm membranes, and injected (10 μL isocratic, 5 μL gradient).

3.2. System suitability

Following method optimization, system suitability experiments were applied to confirm the reliability and reproducibility of the chromatographic system. Initially, slight intentional variations were introduced in critical chromatographic parameters such as column temperature and flow rate to assess method robustness. Subsequently, system suitability parameters were evaluated. The key performance metrics consisted of retention time (min), theoretical plates, asymmetry, capacity factor (k'), and resolution [14].

3.3. Method validation and calibration curves

Validation was performed in compliance with ICH Q2 (R2) for linearity, accuracy, precision, specificity, and robustness [15].

- **Isocratic Method:** Calibration ranges for DTG (1.0–100.0 $\mu\text{g}/\text{mL}$); for 3TC (1.0–300.0 $\mu\text{g}/\text{mL}$)
- **Gradient Method:** Calibration ranges for DTG (2.0–100.0 $\mu\text{g}/\text{mL}$); 3TC (5.0–600.0 $\mu\text{g}/\text{mL}$), and ABA (10.0–600.0 $\mu\text{g}/\text{mL}$)

Limit of detection (LOD) and limit of quantitation (LOQ) values were calculated based on the standard deviation of the response and slope, as per ICH guidelines [15].

3.4. Analysis of laboratory-prepared mixtures

Binary (3TC and DTG) and ternary (3TC, DTG, ABA) mixtures were prepared across their calibration ranges. Specific volumes of stock solutions were transferred to 5-mL volumetric flasks, diluted with the respective mobile phase, and filtered through a 0.22 μm membrane filter. Samples were injected (10 μL isocratic, 5 μL gradient) in triplicate and quantified using their respective calibration equations.

3.5. Application to pharmaceutical preparations

The validated HPLC techniques were applied to the analysis of both *Triumeq*[®] film-coated tablets contain a fixed-dose combination of 50 mg DTG, 300 mg 3TC, and 600 mg ABA and in-house pharmaceutical products containing 50 mg DTG and 300 mg 3TC per tablet. For each product, ten tablets were accurately weighed, powdered, and a portion equivalent to one tablet was transferred into a 50 mL volumetric flask, then proceeded as follows:

For *Triumeq*[®] tablets: 30 mL of a gradient mobile phase (50:50 v/v) was added, and the mixture was sonicated for 25 min to ensure complete drug extraction. The solution was then brought to volume with the same mobile phase and filtered through a 0.22 µm membrane filter. Aliquots were appropriately diluted to prepare final concentrations of 25.0 µg/mL DTG, 150.0 µg/mL 3TC, and 300.0 µg/mL ABA.

For the in-house formulation of DTG/3TC: 30 mL of methanol was added, and the mixture was sonicated for 25 min to ensure complete drug extraction. The solution was then brought to volume with the isocratic mobile phase and filtered through a 0.22 µm membrane filter. Aliquots were appropriately diluted to prepare final concentrations of 25.0 µg/mL DTG and 150.0 µg/mL 3TC. All specimens were analyzed using their respective validated HPLC methods.

4. Results and discussion

4.1. AI-assisted method scouting across diverse engines and the indispensable role of human expertise (human-in-the-loop)

Integration of diverse artificial intelligence engines into our field represents a significant enrichment of chemical analysis. While these engines may propose various methodologies, their true value lies in their ability to inspire experimental application and iterative refinement. By practically implementing AI-generated suggestions and optimizing them for efficiency and accuracy, we unlock the potential for an infinite array of analytical approaches. This synergy between human expertise (human-in-the-loop) and machine intelligence not only accelerates discovery but also expands the boundaries of what is analytically possible. Therefore, while AI engines may propose similar or alternative methodologies, their integration into analytical workflows must always be accompanied by thorough method validation. This ensures that AI serves as a complementary asset, not a substitute, in the pursuit of robust and efficient analytical solutions.

AI was used in this study as an expected method scouting tool to propose probable starting conditions for HPLC development (e.g., stationary phase type, organic modifier, pH range, elution mode, and a general UV wavelength). However, AI outputs are not equivalent to a validated analytical method. They are best viewed as hypothesis-generating predictions that require (i) chromatographic reasoning by an expert analyst and (ii) experimental verification under real instrumental constraints.

To demonstrate transparency and reproducibility, the same standardized prompt was submitted to multiple widely used AI engines (**Microsoft Copilot, ChatGPT 5.2, Google Gemini, and Perplexity**),. **Standardized prompt used across various AI engines** as follows:

“Suggest two HPLC methods: one isocratic for binary analysis of dolutegravir (DTG) and lamivudine (3TC), and one gradient method for ternary analysis of DTG, 3TC, and abacavir (ABA), in pure and pharmaceutical formulation. I need to measure concentration using HPLC-UV. Please include column type, mobile phase, flow rate, elution type, and detection settings.”

Across different AI engines, the most consistent suggestions were: (a) use of RP-C18 as stationary phase, (b) working at acidic pH (~3) to improve peak shape for basic/ionizable analytes, and (c) UV detection in the ~254–260 nm region as a compromise wavelength. Nevertheless, the AI engines frequently proposed longer run times, non-optimal

organic modifiers, or generic single-wavelength detection, and they did not reliably anticipate peak tailing behavior or practical constraints such as column availability and the need for rapid routine quality control.

As detailed in [Table 1.a](#) and [b](#), the Copilot tool provided comprehensive and reproducible parameters, including column dimensions, flow rate, UV wavelength, and clear. This facilitated better resolution and greater control over the method, making it easier to implement and refine. In contrast, other AI engines often omitted key details or offered vague suggestions, resulting in overlapping or compressed retention windows that increased the risk of co-elution and poor peak resolution. Therefore, in this work, we utilized the Copilot tool because it supports experimental reasoning by offering practical, testable suggestions that can be refined in the laboratory. In summary, Copilot excels in scientific depth, practical guidance, and adaptability, making it a valuable partner helpful for starting-point prediction, whereas human expertise and experimental optimization remain essential for robust chromatographic method development. To enable a direct visual comparison between AI scouting and the final optimized methods, chromatograms obtained using the initial Copilot-suggested conditions were compared with chromatograms obtained under the final optimized conditions. These comparisons are provided in **Supplementary Fig. S1(a–d): panels (a) and (b)** show the binary mixture (3TC/DTG) under the final optimized isocratic conditions and the Copilot-suggested scouting conditions, respectively, while **panels (c) and (d)** show the ternary mixture (3TC/ABA/DTG) under the final optimized gradient conditions and the Copilot-suggested scouting conditions, respectively. The results illustrate that Copilot suggestions supported rapid selection of initial conditions, whereas experimental optimization and analyst expertise were required to achieve the final validated methods. Therefore, the final methods were obtained through a **human-in-the-loop** workflow, where the analyst applied domain knowledge to translate AI outputs into a robust, green, and fit-for-purpose method. Key expert-driven refinements included:

- 1. Peak-shape correction by additive selection:** DTG exhibited tailing under initial scouting conditions. The addition of TEA (silanol masking) and operation at low pH were selected based on chromatographic principles to reduce secondary interactions and improve symmetry.
- 2. Column selection aligned to the separation problem:** While most engines suggested a conventional 250 mm C18 column, experimental optimization showed that a shorter 100 mm ODS2 column combined with a steep, short gradient achieved baseline separation for the ternary mixture with markedly reduced run time and solvent waste.
- 3. Mobile phase optimization beyond “generic suggestions”:** AI engines frequently suggested methanol-rich isocratic systems for the binary mixture. Experimentally, ACN–phosphate buffer (pH 3.5) offered superior efficiency and DTG peak symmetry, enabling $\alpha < 5$ min run time while maintaining resolution.
- 4. Compound-specific wavelength selection using PDA:** AI engines commonly proposed a single UV wavelength (≈ 260 nm). In contrast, PDA-based evaluation identified compound-specific maxima and improved sensitivity using 258 nm (DTG), 275–278 nm (3TC), and 294 nm (ABA), enhancing quantitation while maintaining selectivity.

Overall, these observations support the central concept of this manuscript: **AI accelerated the early-stage exploration**, but **human expertise and experimental refinement were decisive** in achieving (i) short run time, (ii) acceptable system suitability, (iii) robust performance under small parameter variations, and (iv) alignment with green and white analytical chemistry objectives. Finally, the returned “output parameters” suggested by various AI-engines were then compared with the final experimentally optimized conditions adopted in this work ([Table 1](#)).

Table 1

Comparison of AI-engine suggested HPLC conditions (same prompt) versus final experimentally optimized conditions.

a. Binary (DTG + 3TC) – Isocratic scouting vs final method							
AI-engine	Column suggested	Mobile phase suggested	pH	Flow (mL/min)	Suggested tR (min)	UV (nm)	Main mismatch vs final
Copilot	C18, 250 × 4.6 mm, 5 μm	MeOH: phosphate buffer (≈45:55)	~3.2	1.0	3TC: ~3.5; DTG: ~8.0	260	Longer run; MeOH gave less favorable DTG peak shape vs ACN in our system
ChatGPT 5.2	C18, 250 × 4.6 mm, 5 μm	MeOH: phosphate buffer (≈65:35)	~3.0	1.0	3TC: 2.0–2.8; DTG: 5.0–6.5	260	High MeOH fraction predicted; experimentally ACN improved symmetry and speed
Gemini	C18, 150 × 4.6 mm, 5 μm	MeOH: phosphate buffer (≈45:55)	~3.0	1.0	Not provided	260	Generic wavelength; column length not optimized for our separation objective
Perplexity	C18, 250 × 4.6 mm, 5 μm	MeOH: phosphate buffer (≈70:30)	~3.0	1.0	3TC: 2.5–3.0; DTG: 5.5–6.5	260	Predicted longer method; injection volume generic
Final (in-lab optimized)	XBridge® C18, 250 × 4.6 mm, 5 μm	0.02 M phosphate buffer (pH 3.5): ACN (50:50, v/v) + TEA	3.5	1.2	3TC: 1.967; DTG: 3.720	258 (DTG), 275 (3TC)	Expert changes: ACN + TEA to fix DTG tailing; dual λ from PDA spectra; <5 min runtime
b. Ternary (DTG + 3TC + ABA) – Gradient scouting vs final method							
AI engine	Column suggested	Mobile phase suggested	pH	Flow (mL/min)	Gradient/run time	UV (nm)	Main mismatch vs final
Copilot	C18, 250 × 4.6 mm, 5 μm	A: 0.1 % TFA + 0.05 % TEA; B: MeOH	~acidic	1.0	Multi-step; up to ~15–20 min	260	Too long; not optimized for 6 min target
ChatGPT 5.2	C18, 250 × 4.6 mm, 5 μm	A: phosphate buffer pH ~3; B: ACN	~3.0	1.0	~10 min	257–260	Longer runtime; single λ generic
Gemini	C18, 250 × 4.6 mm, 5 μm	A: acidic water; B: ACN	~2.5–3	1.2	~15 min	254–257	Overly general; not guided by system suitability outcomes
Perplexity	C18, 150 × 4.6 mm, 5 μm	A: phosphate buffer pH ~3; B: mixed organic	~3.0	1.0	~15 min	~257	Longer runtime; less emphasis on rapid re-equilibration
Final (in-lab optimized)	Spherisorb® ODS2 C18, 100 × 4.6 mm, 3.5 μm	A: 0.175 % TEA + 0.1 % TFA in water (pH 3.15); B: MeOH	3.15	1.2	0–2.0 min 50:50; 2.1–4.0 min 0:100; 4.2–6.0 min 50:50	258 (DTG), 278 (3TC), 294 (ABA)	Expert changes: shorter column + short gradient for ≤6 min; multi-λ for sensitivity; ensured baseline separation

4.2. Copilot as the AI-based prediction tool used in this study (ex-ante scouting)

In this study, Microsoft Copilot was used as an AI-based method scouting (prediction) tool to generate initial chromatographic conditions based on the analytes' physicochemical properties and the intended analytical purpose. The AI-generated suggestions were then experimentally applied, refined in the laboratory, and fully validated to ensure method suitability for routine quality control.

The Copilot-assisted workflow included the following steps:

- **AI tool used:** Microsoft Copilot (web-based), <https://copilot.microsoft.com>.
- **Input parameters:** analytes (DTG, 3TC, and/or ABA), sample type (pure standards and pharmaceutical formulations), analytical objective (assay/quantification), detector type (HPLC–UV/PDA), and relevant physicochemical characteristics (e.g., polarity, pKa, solubility, molecular weight).
- **Prompt used for AI prediction:** a standardized prompt detailed in Section 4.1.
- **AI output parameters:** proposed column characteristics, mobile phase composition/ratios, pH range, flow rate, elution type (isocratic/gradient), and suggested UV wavelength(s).
- **Experimental application:** the AI-suggested conditions were implemented as **initial scouting experiments** to evaluate retention behavior, peak shape, and separation performance.
- **In-lab refinement:** chromatographic parameters (e.g., organic modifier, pH, additive use, gradient program, flow rate, and detection wavelength) were systematically adjusted to achieve optimum separation, improved peak symmetry, and reduced run time.
- **Validation:** the final optimized methods were validated according to ICH Q2(R2) to ensure transparency, accuracy, robustness, and reproducibility of the AI-assisted methodology.

4.3. The isocratic HPLC technique for simultaneous determination of DTG and 3TC

4.3.1. AI-driven isocratic HPLC method development for DTG and 3TC

Initial chromatographic conditions for the simultaneous determination of DTG and 3TC were generated using an AI-Copilot tool. After ask by previous prompt mention in Section 4.1.

Output parameters: the suggested output parameters are summarized in Text Box 1.

Under the proposed AI-driven conditions, preliminary experiments yielded retention times of approximately 3.5 min for lamivudine (3TC) and 8.0 min for dolutegravir (DTG). However, initial trials showed significant tailing for DTG, indicating suboptimal peak symmetry and potential issues with analyte-stationary phase interactions. To address this, laboratory-based optimization was undertaken. A series of systematic modifications were applied to fine-tune the composition of the mobile phase, pH, and flow rate, in order to enhance peak shape, reduce tailing, and improve resolution. These iterative adjustments resulted in a robust and reproducible isocratic HPLC method with acceptable system suitability parameters for both analytes.

4.3.2. Refinement of AI-driven (in-silico) isocratic HPLC method for DTG and 3TC

Following the initial AI-generated method, in-laboratory refinement was performed to optimize chromatographic performance and ensure the method's suitability for routine application. The final selected system parameters are given in Table 2.

Chromatographic separation of DTG and 3TC was achieved using an XBridge® C18 column (5 μm, 4.6 × 250 mm). The optimized mobile phase, including phosphate buffer (pH 3.5) and acetonitrile in a 50:50 v/v ratio, was delivered at a flow rate of 1.2 mL/min (as detailed in Table 2).

The substitution of methanol with acetonitrile (ACN) significantly improved resolution and peak symmetry, particularly benefiting DTG

Text Box 1

Chromatographic conditions.

Text Box 1**Column & Conditions**

- Column: C18 (250 mm × 4.6 mm, 5 μm particle size)
- Flow Rate: 1.0 mL/min
- Injection Volume: 10 μL
- Detection Wavelength: 260 nm (suitable for both DTG & 3TC)
- Column Temperature: 30 °C

Mobile Phase Composition (Isocratic)

- Methanol: 45%
- Phosphate Buffer (20 mM, pH 3.2): 55%
- *Note: Buffer pH optimized to enhance DTG retention while maintaining 3TC resolution*

Table 2
Chromatographic conditions of the proposed HPLC methods.

Parameter	Isocratic Method	Gradient Method
Analyte	DTG and 3TC	DTG, 3TC, and ABA
Analytical Column	Waters XBridge® C18, 5 μm, 4.6 × 250 mm	Waters Spherisorb® ODS2 C18, 3.5 μm, 4.6 × 100 mm
Mobile Phase	0.02 M Phosphate Buffer (pH 3.5): ACN (50:50, v/v)	A: 0.175 % TEA + 0.1 % TFA in water B: Methanol
Program	Isocratic	0.0–2.0 min: A/B = 50:50 v/v 2.1–4.0 min: A/B = 0:100v/v 4.2–6.0 min: A/B = 50:50v/v
Flow Rate	1.2 mL/min	1.2 mL/min
Injection Volume	10.0 μL	5.0 μL
Column Temperature	35 °C	40 °C
Autosampler Temperature	–	20 °C
Runtime	5.0 min	6.0 min
Wavelengths (λ)	λ ₁ : 258 nm (DTG), λ ₂ : 275 nm (3TC)	λ ₁ : 258 nm (DTG), λ ₂ : 278 nm (3TC), λ ₃ : 294 nm (ABA)

because of its lipophilic character. Incorporation of TEA in the mobile phase effectively masked residual silanol groups on the stationary phase, thereby minimizing peak tailing, especially for DTG. The XBridge C18 column, known for its high loading capacity and column efficiency, provided robust performance even with complex sample matrices.

The selected 50:50 isocratic elution offered an optimal balance between separation efficiency and analysis time, making the method well-suited for high-throughput or routine quality control environments.

Under these refined conditions, the retention times for 3TC and DTG were 1.967 min and 3.720 min, respectively. Detection wavelengths were optimized at 275 nm for 3TC and 258 nm for DTG, providing greater sensitivity than the initially suggested single wavelength of 260 nm by the AI model. All standard and sample solutions were injected with a volume of 10.0 μL.

4.3.3. Evaluation of system suitability for the isocratic method under variable chromatographic conditions

System suitability testing was performed to evaluate the robustness and performance reliability of the applied isocratic HPLC Method (DTG and 3TC) under deliberately altered conditions, including variations in detection wavelength, column temperature, mobile phase composition, and flow rate. The results in **Table 1S** indicated consistent system performance across all tested parameters:

- **Wavelength Variation** (256–260 nm for DTG, 273–277 nm for 3TC): Retention times, asymmetry, theoretical plates, and capacity factors for both DTG and 3TC showed minimal variation. Peak areas varied slightly but remained within acceptable limits, confirming detector response stability.
- **Column Temperature** (33–37 °C): No significant shifts in retention time or asymmetry were observed. For DTG, the plate number increased slightly with rising temperature, indicating better efficiency at higher temperatures. 3TC also showed stable performance across the range.
- **Mobile Phase Ratio**: Adjusting the acetonitrile/phosphate buffer (pH 3.5) ratio impacted retention times as expected higher organic content (55:45 v/v) resulted in slightly affects retention for DTG and 3TC. Despite this, peak symmetry and plate count remained consistent, indicating the robustness of the method.
- **Flow Rate Variation** (1.1–1.3 mL/min): Changes in flow rate inversely affected retention time for both analytes, with higher flow decreasing retention. However, plate numbers and symmetry factors were unaffected, indicating good method robustness.

These results confirm the efficiency of the proposed isocratic HPLC technique for the separation binary mixture under small variations in the suggested operational conditions.

4.3.4. System suitability parameters of the proposed isocratic HPLC technique under optimized conditions

System suitability experiments were conducted to ensure the adequacy of the proposed isocratic HPLC method for routine analysis. The chromatographic system demonstrated excellent performance under optimized conditions, as evidenced by the following parameters:

- **Resolution (R_s)** between DTG and 3TC exceeded the acceptance threshold of 2.0, confirming satisfactory peak separation and method selectivity.
- **Tailing Factors** for both analytes were consistently below 1.5, indicating symmetrical peak shapes. The use of ACN as the organic modifier contributed to improved peak symmetry and reduced tailing due to its higher elution strength and favorable interaction with analyte functional groups.
- **Theoretical Plate Numbers (N)** surpassed 5000 for both DTG and 3TC, reflecting high column efficiency and reproducibility of the method.

Collectively, these parameters confirm that the technique meets system suitability requirements for quantitative analysis. The detailed results are presented in Table 3 and Fig. 2.

4.4. Gradient HPLC technique for a ternary mixture for simultaneous separation of DTG, 3TC, and ABA

4.4.1. AI-driven (in-silico) gradient HPLC method for DTG, 3TC, and ABA

Initial chromatographic parameters for the simultaneous separation of DTG, 3TC, and ABA were generated using an AI-Copilot tool. After ask by previous prompt mention in Section 4.1.

Output parameters: The suggested output parameters are summarized in (Text Box 2).

The proposed gradient method generated by the AI tool resulted in a total run time of approximately 15 min. However, preliminary laboratory trials revealed that the AI-recommended gradient method had critical limitations in system suitability, including an extended run time and suboptimal resolution. Consequently, in-lab experimental refinement was undertaken to enhance the efficiency and performance of the method, employing a systematic approach to optimize the method.

4.4.2. Refinement of AI-driven (in-silico) gradient HPLC method for DTG, 3TC, and ABA

To overcome the limitations of the initial in-silico approach, systematic in-lab refinement was undertaken to develop a more robust and time-efficient gradient HPLC method. The optimized method employed a Spherisorb ODS2® C18 column (3.5 μm , 4.6 \times 100 mm) and a gradient mobile phase system including methanol, TEA, and TFA buffer at pH 3.15.

The gradient program was as follows:

- **0–2.0 min:** Methanol: TFA buffer (50:50, v/v)
- **2.1–4.0 min:** 100 % methanol

- **4.2–6.0 min:** Re-equilibration with Methanol:TFA buffer (50:50, v/v)

This elution strategy enabled efficient separation of the three analytes with clear baseline resolution. Detection was performed at compound-specific absorbance maxima: 258 nm for DTG, 278 nm for 3TC, and 294 nm for ABA.

The method employed a flow rate of 1.2 mL/min and achieved retention times of 1.210 min (3TC), 1.480 min (ABA), and 4.103 min (DTG), as shown in Fig. 3.

This gradient system offers significant advantages:

- **Methanol:** Provides acceptable elution strength and selectivity with improved environmental compatibility compared with acetonitrile.
- **TEA:** Acts as a silanol blocker, reducing secondary interactions and minimizing peak tailing, particularly for lipophilic or basic drugs such as DTG and ABA.
- **TFA buffer (pH 3.15):** Maintains a low-pH environment, suppressing the ionization of basic functional groups, which enhances retention and improves peak symmetry. Additionally, TFA is generally compatible with UV detection at the selected wavelengths.

The gradient design ensures effective separation of compounds with varying polarities: early elution for polar 3TC, intermediate retention for moderately polar ABA, and delayed elution for highly lipophilic DTG; thus overcoming the limitations of isocratic methods for ternary mixtures.

4.4.3. Evaluation of system suitability under variable chromatographic conditions

To assess the robustness and reliability of the developed Gradient HPLC technique, system suitability testing was performed under intentionally varied chromatographic conditions. Parameters examined included detection wavelength, column temperature, mobile phase composition, and flow rate.

The results, summarized in Table 2S, demonstrated minimal variation across all tested parameters:

- **Wavelength (256–260 nm):** Retention times, symmetry factors, and theoretical plate counts for both DTG and 3TC remained stable, indicating wavelength tolerance without compromising sensitivity or peak integrity.
- **Column Temperature (33–37 °C):** Minor improvements in the theoretical plate counts were observed with increasing temperature, particularly for DTG, while retention times and peak symmetry remained consistent.
- **Mobile Phase Ratio:** a minor change in the ratio of methanol: TFA buffer (50:50, v/v) resulted in variations in retention times corresponding to changes in organic content; however, symmetry and resolution were maintained, and all peaks remained well-separated.
- **Flow Rate (1.1–1.3 mL/min):** Increasing flow rates resulted in reduced retention times, but no deterioration in peak shape or resolution was observed.

Table 3

System suitability parameters for DTG and 3TC using the isocratic HPLC technique, and for DTG, 3TC, and ABA using the gradient technique.

Compound	HPLC Method	Retention Time (min)	Theoretical Plates	Asymmetry	Capacity Factor (k')	Resolution
DTG	Isocratic	3.720	15,597	1.10	17.60	–
	Gradient	4.103	49,675	1.21	38.84	32.8
3TC	Isocratic	1.967	8481	1.10	8.84	16.5
	Gradient	1.210	3911	1.13	10.78	–
ABA	Gradient	1.480	4508	0.95	13.40	3.26

Resolution (R_s) values were calculated for the peak pairs reported in the table; in the gradient method, $R_s = 3.26$ corresponds to 3TC/ABA and $R_s = 32.8$ corresponds to 3TC/DTG.

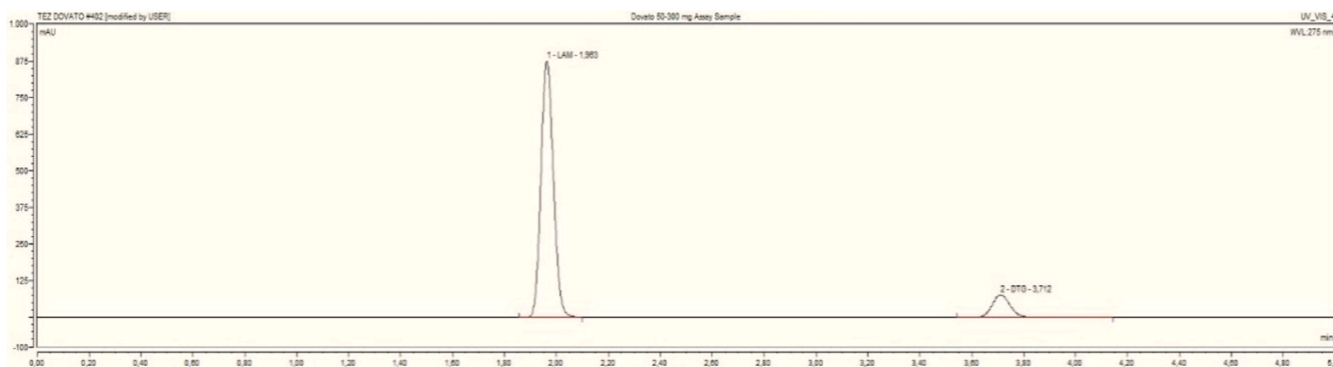


Fig. 2. Chromatogram of 3TC and DTG using the isocratic HPLC method.

Text Box 2

AI assisted chromatographic conditions.

Text Box 2

Column:

C18, 250 mm × 4.6 mm, 5 μm particle size

Mobile Phase Composition:

- Mobile Phase A: 0.1% TFA + 0.05% TEA in water
- Mobile Phase B: Methanol

Gradient Program: 85 : 15 v/v (0.0) , 60:40 v/v (0.1- 5.0 min) , 40:60 v/v (5.1-10.0) , 20:80 v/v (10.1 -15.0 min) and 85 : 15 v/v (15.1-20.0 min)

Flow Rate: 1.0 mL/min

Detection: UV at 260 nm

Column Temp: 30 °C

Injection Volume: 5 μL

These findings confirm the robustness of the method, as all system suitability parameters remained within acceptable limits even under stressed conditions.

4.5. System suitability parameters of the proposed gradient HPLC technique under optimized conditions

System suitability testing was performed under the optimized

gradient chromatographic conditions to confirm that the proposed gradient RP-HPLC method is suitable for routine simultaneous determination of 3TC, ABA, and DTG. The evaluated parameters included retention time (min), theoretical plates (N), asymmetry, capacity factor (k'), and resolution (R_s), as summarized in [Table 2](#).

Under the optimized gradient conditions, the retention times were 1.210 min for 3TC, 1.480 min for ABA, and 4.103 min for DTG. The method showed acceptable column efficiency and peak symmetry, with

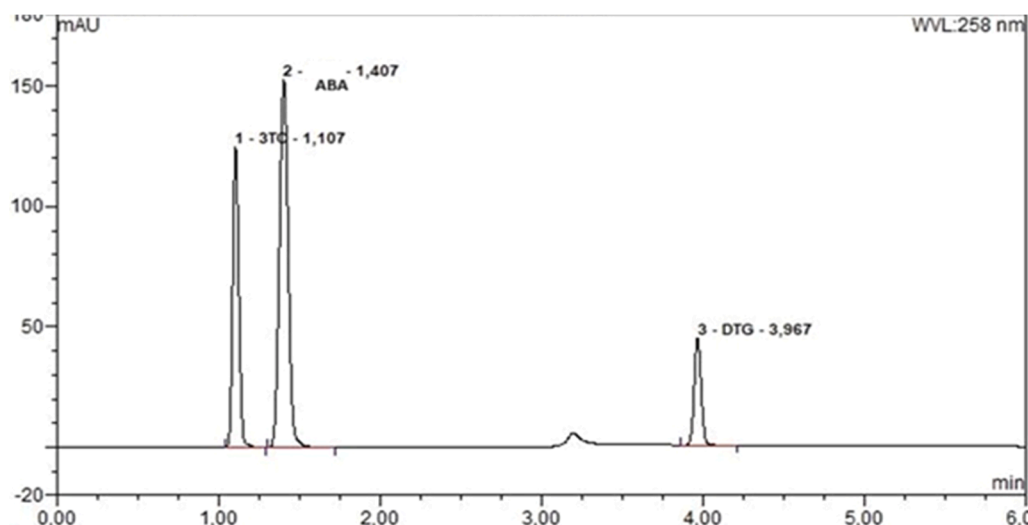


Fig. 3. Chromatogram of 3TC, DTG and ABA using the gradient HPLC method.

theoretical plate numbers of 3911 (3TC), 4508 (ABA), and 49,675 (DTG), and asymmetry values of 1.13 (3TC), 0.95 (ABA), and 1.21 (DTG). Adequate chromatographic separation was obtained, where the resolution between ABA and 3TC was 3.26, and the resolution between DTG and 3TC was 32.8 (Table 2). Collectively, these results confirm that the proposed gradient HPLC technique provides efficient separation with reproducible performance under the optimized conditions and is suitable for routine analysis.

Under these conditions, the total run time was 6.0 min, significantly shorter than previously reported methods (e.g., 10 min in reference [7]), thereby enhancing throughput and analytical economy. These results are illustrated in Fig. 3, confirming that the technique is both efficient and suitable for routine quality control applications.

4.6. Validation of the isocratic and gradient HPLC techniques

Validation of both the isocratic and gradient HPLC techniques was performed by the ICH Q2(R2) guideline [15]. Parameters assessed included linearity, accuracy (recovery), precision, and robustness. The obtained results confirm that the developed techniques are suitable for the quantitative analysis of 3TC, DTG, and ABA in their pharmaceutical formulations.

4.6.1. Linearity, LOD, and LOQ

For the isocratic HPLC technique, calibration graphs were established over the concentration ranges of 1.0–100.0 µg/mL for DTG and 1.0–300.0 µg/mL for 3TC. For the gradient RP-HPLC method, the calibration ranges were 2.0–100.0 µg/mL for DTG, 5.0–600.0 µg/mL for 3TC, and 10.0–600.0 µg/mL for ABA.

The linearity of the proposed methods was evaluated by analyzing a series of standard solutions at multiple concentration levels for each analyte. Each concentration was injected in triplicate to ensure statistical reliability. All analytes indicated excellent linearity, with correlation coefficients (*r*) exceeding 0.999, indicating a strong linear relationship across the tested ranges. The regression equations and corresponding (*r*) values were presented in Table 4. The limits of detection (LOD) and the limits of quantification (LOQ) for each compound were calculated, and the values are provided in Table 4.

4.6.2. Accuracy (Recovery studies)

The accuracy of the technique was evaluated through recovery experiments by spiking standard analyte solutions at three concentration levels: 80 %, 100 %, and 120 % of the target concentration for each of the three drugs. The recovery percentages were found to be within the

Table 4

Validation parameters for DTG and 3TC using the isocratic HPLC method, and for DTG, 3TC, and ABA using the gradient method.

Parameter	HPLC Method	DTG	3TC	ABA
Concentration range (µg/mL)	Isocratic	1.0–100.0	1.0–300.0	–
	Gradient	2.0–100.0	5.0–600.0	10.0–600.0
Wavelength (nm)	Isocratic	258	275	–
	Gradient	258	278	294
Intercept	Isocratic	–0.0530	0.1293	–
	Gradient	–0.19231	1.34847	3.1178
Slope	Isocratic	0.4902	0.3140	–
	Gradient	0.201830	0.192533	0.351516
Correlation coefficient (<i>r</i>)	Isocratic	1.0000	1.0000	–
	Gradient	0.9997	0.9991	0.9958
Accuracy (Mean ± SD)	^a Isocratic	98.47 ± 0.65	101.17 ± 0.83	–
	^b Gradient	99.80 ± 0.80	100.40 ± 0.36	99.83 ± 0.93
Precision	Isocratic	1.15	0.98	–
	Gradient	1.57	1.13	–
Precision	Isocratic	1.85	0.85	0.76
	Gradient	1.95	1.63	1.14
LOD (µg/mL)	Isocratic	0.31	0.28	–
	Gradient	0.25	0.59	0.96
LOQ (µg/mL)	Isocratic	0.94	0.84	–
	Gradient	0.83	1.96	3.17

^a Average RSD of freshly prepared solutions in three various concentrations was repeated 8 times on the same day.

^b Average RSD of freshly prepared solutions in three various concentrations, repeated 5 times over five days.

acceptable range, with values spanning from 98.47 % to 101.17 %. These results confirm the accuracy and reliability of the technique for the simultaneous quantification of the analytes.

4.6.3. Precision

Intra-day and inter-day precision were evaluated by analyzing specimen sets at three concentration levels, in triplicate. For intra-day testing, specimens were kept at 20 °C and analyzed on the same day. For inter-day analysis, specimens were stored at 20 °C and in dark conditions over five consecutive days. Precision, with all values found to be below 2.0 % as shown in Table 4, confirms the repeatability and intermediate precision of the method. The obtained results for the

isocratic HPLC method are presented in Tables 4S and 5S, and the results for the gradient HPLC method are given in Tables 6S and 7S.

4.6.4. Specificity

Specificity evaluates the method's ability to accurately quantify each analyte in the presence of other substances, such as excipients or co-formulated drugs. The specificity of the two proposed HPLC methods was thoroughly examined using laboratory-prepared mixtures and a commercial formulation.

The isocratic HPLC technique, developed for the simultaneous quantification of 3TC and DTG, was applied to synthetic mixtures containing these two drugs in various ratios as well as their In-house formulation. As shown in Table 8S, both analytes were successfully separated and quantified without interference, with percentage recoveries ranging from 99.90 % to 100.28 % and standard deviations within acceptable limits. These results confirm the specificity of the method for binary mixtures.

The gradient HPLC technique was developed to simultaneously determine 3TC, DTG, and ABA in marketed Triumeq® tablets. A variety of ternary mixtures with different proportions of the three drugs were analyzed, and recoveries for all analytes ranged from 99.74 % to 100.34 %, indicating excellent resolution and no matrix interference (Table 5). Furthermore, the method was directly applied to Triumeq® tablets (containing DTG 50 mg, 3TC 300 mg, and ABA 600 mg), where all three analytes were accurately quantified.

To validate these results and rule out matrix effects, the standard addition technique was employed. As shown in Table 5, recoveries for all drugs in Triumeq® ranged from 99.88 % to 100.21 %, confirming that both methods are highly specific and suitable for routine quality control of 3TC, DTG, and ABA in both laboratory-prepared mixtures and pharmaceutical formulations.

4.6.5. Robustness

Robustness of the proposed HPLC techniques was evaluated by intentionally varying key chromatographic parameters within realistic operational ranges: wavelength ± 2 nm, column temperature ± 2 °C, mobile phase organic content ± 2 %, and flow rate ± 0.1 mL/min. The impact of these variations was assessed on key system suitability metrics, including retention time, asymmetry factor, number of theoretical plates (N), capacity factor (k'), and peak area. The method remained unaffected by these small deliberate changes, confirming its robustness and reliability for routine applications. The observed values are summarized in Tables 1S and 2S.

Table 5

Quantification of the active ingredient in Triumeq® Tablets and in-house formulation by the two proposed HPLC methods and application of the standard addition technique.

Parameter	Isocratic HPLC Method		Gradient HPLC Method		
	3TC	DTG	3TC	DTG	ABA
Drug					
Label Claim (mg)	300.0	50.0	300.0	50.0	600.0
% Found ^a \pm SD	100.40 ± 0.10	100.50 ± 0.40	100.40 ± 0.17	100.20 ± 0.61	99.40 ± 0.45
Standard addition ^b Mean \pm SD	99.82 \pm 0.22	100.50 \pm 0.12	99.22 \pm 0.22	100.22 \pm 0.35	100.11 \pm 0.31

^a Average of three determinations.

^b Results represent the average of three replicate experiments. For the isocratic HPLC method, pure standards were added at concentrations equivalent to 10.0, 20.0, and 30.0 μ g/mL to a sample containing 150.0 μ g/mL of 3TC, and at 5.0, 10.0, and 20.0 μ g/mL to a sample containing 25.0 μ g/mL of DTG. For the gradient HPLC method, pure standards were added at concentrations equivalent to 10.0, 20.0, and 30.0 μ g/mL to a sample containing 300.0 μ g/mL of ABA.

4.7. Application of the suggested method to the formulations

The validated HPLC methods were successfully applied to the assay of commercially available pharmaceutical products. The gradient RP-HPLC methods was used for the simultaneous quantification of DTG, 3TC, and ABA in Triumeq® film-coated tablets, while the isocratic HPLC method was employed for the quantification of DTG (50 mg) and 3TC (300 mg) in an in-house fixed-dose combination formulation (50/300 mg DTG/3TC tablets). The assay results demonstrated that the content of each API was within acceptable ranges. Furthermore, no interfering peaks from excipients were observed, confirming the specificity of both methods. Detailed assay results are provided in Table 4.

4.8. Statistical analysis

Statistical analysis was performed to evaluate the reliability and accuracy of the proposed isocratic and gradient HPLC methods by comparing their results with those obtained using previously reported methods [6,8]. The comparison was conducted using pure powdered forms of 3TC, DTG, and ABA. Student's *t*-test and *F*-test were applied to assess any significant differences between the proposed and reported techniques. As shown in Table 6, the calculated *t* and *F* values were lower than the corresponding critical values at the 95 % confidence level ($P = 0.05$), indicating no statistically significant difference between the two sets of results.

5. Smart analytical method alignment and comparative evaluation

The transformation of analytical chemistry into a more sustainable and innovative discipline is driven by the principles of Green Analytical Chemistry (GAC), White Analytical Chemistry (WAC), and recent advancements in digital technologies. In line with this paradigm shift, the proposed isocratic and gradient HPLC methods were developed using AI-assisted optimization to improve chromatographic performance while ensuring environmental sustainability and regulatory compliance.

These methods demonstrated significant improvements over previously reported approaches in terms of analytical throughput, solvent efficiency, and environmental impact. The isocratic method operates at a flow rate of 1.2 mL/min with a total runtime of 5 min, generating only 6.0 mL of solvent waste and enabling approximately 11 analyses per hour. Similarly, the gradient method runs at 1.2 mL/min over 6.0 min, producing 7.2 mL of waste and achieving 10 analyses per hour. These figures represent a substantial reduction in solvent consumption; down to 58 %; compared to conventional methods that typically require up to 17 min per run with waste volumes as high as 17 mL, thereby nearly doubling the analytical throughput.

The greenness of the developed methods was quantitatively evaluated using the AGREE metric, resulting in scores of 0.64 for the isocratic method and 0.65 for the gradient method. Additionally, both methods achieved a whiteness score of 75 based on WAC criteria, confirming their balanced performance across analytical efficiency, environmental sustainability, and practical applicability. These high GAC and WAC scores underscore the suitability of the methods for integration into modern quality control workflows and sustainable pharmaceutical analysis. The summarized results are presented in Table 7.

From a regulatory perspective, both techniques were validated in accordance with the latest ICH Q2(R2) guidelines [15], ensuring their robustness, reproducibility, and suitability for purpose. Additionally, the incorporation of AI in the method development process accelerated optimization, enhancing efficiency and precision.

In summary, the proposed HPLC methods qualify as smart analytical techniques that are AI-driven, eco-efficient, time-saving, and compliant with ICH Q2(R2) guidelines [15]. Their capacity to minimize waste, maximize analytical throughput, and meet modern regulatory standards makes them ideal for routine pharmaceutical quality control.

Table 6

Statistical comparison between the results obtained by the proposed HPLC methods and the reported methods for analysis of 3TC, DTG, and ABA in their pure powdered forms.

Parameter	3TC		DTG		ABA	
	Proposed Method	Reported Method [6] ^a	Proposed Method ^a	Reported Method [6] ^a	Proposed Method	Reported Method [6] ^a
Isocratic HPLC Method						
Mean (%)	99.72	99.85	100.08	99.62	—	—
± SD	0.42	0.60	0.51	0.40	—	—
n	6	6	6	6	—	—
Variance	0.176	0.360	0.260	0.160	—	—
Student's <i>t</i> -test ^c	0.434 (2.228)	—	1.738 (2.228)	—	—	—
F-test ^c	2.045 (5.05)	—	1.625 (5.05)	—	—	—
Gradient HPLC Method						
	Proposed Method	Reported Method [8] ^a	Proposed Method ^a	Reported Method [8] ^a	Proposed Method	Reported Method [8] ^a
Mean (%)	99.65	100.03	100.10	99.92	99.86	99.45
± SD	0.38	0.81	0.77	0.48	0.74	0.45
n	6	6	6	6	6	6
Variance	0.144	0.656	0.593	0.230	0.548	0.203
Student's <i>t</i> -test ^c	1.045 (2.228)	—	0.486 (2.228)	—	1.159 (2.228)	—
F-test ^c	4.556 (5.05)	—	2.578 (5.05)	—	2.70 (5.05)	—

^b Reported method refers to the gradient HPLC for the ternary mixture (3TC, DTG, and ABA).

^a Reported method refers to the isocratic HPLC for the binary mixture (3TC and DTG).

^c The figures in parentheses are the corresponding theoretical values at $P = 0.05$.

Furthermore, their design and implementation align with the objectives of the UN Sustainable Development Goals (UN-SDGs), providing a forward-looking model for next-generation chromatographic [16–19].

To further elucidate their advantages, we compared the AI-assisted HPLC methods with conventional HPLC approaches developed without AI support, using a tabulated structure for result presentation, following the format reported in references [20–27]. The evaluation framework shown in Table 7 encompassed the following criteria:

- Red (R): Method performance (scope, sensitivity, precision, accuracy)
- Green (G): Environmental sustainability (AGREE score and runtime)
- Blue (B): Practical aspects (cost-efficiency, time-efficiency, portability)
- White (W): Overall sustainability balance, integrating R, G, and B
- Smart (S): Conformance with GAC and WAC principles through the application of AI

6. Conclusion

The proposed HPLC techniques were successfully applied for the simultaneous quantification of DTG–3TC and DTG–3TC–ABA in a combined in-house formulation, as well as in the commercial triple combination, Triumeq®. The recommended procedures and sample preparation methods proved to be rapid, environmentally friendly, and accurate, requiring no prior derivatization. Method evaluation using penalty points demonstrated that these approaches outperform existing methods in terms of both analytical performance and greenness. These novel HPLC techniques provide improved quantification of DTG, ABA, and 3TC, with short analysis times that enhance environmental sustainability. The methods are fast, cost-effective, and suitable for the routine simultaneous determination of these antiviral drugs. By integrating principles of smart analytical chemistry; combining GAC, WAC, and AI-driven optimization; these HPLC methods represent next-generation tools for sustainable pharmaceutical analysis. They deliver robust performance while minimizing solvent consumption and analysis time, aligning with eco-efficiency and quality assurance objectives in routine quality control.

Using multiple AI engines (Copilot, ChatGPT 5.2, Gemini, and Perplexity) accelerated early method scouting, while final chromatographic conditions were established through a human-in-the-loop workflow involving experimental refinement and ICH Q2(R2) validation. This confirms that AI served as a supportive tool for generating initial

hypotheses, whereas expert judgment and in-lab verification were essential to achieve the final robust and sustainable methods.

Importantly, this study supports the United Nations Sustainable Development Goals (UN-SDGs), contributing to SDG 3 (Good Health and Well-Being), SDG 9 (Industry, Innovation, and Infrastructure), and SDG 12 (Responsible Consumption and Production). Additionally, the research exemplifies SDG 17 (Partnerships for the Goals) through international, women-led collaboration between scientists from Turkey, Egypt, and Syria, advancing sustainable and inclusive science.

7. Future recommendation

Future work should further advance **green analytical chemistry** within an **AI-assisted, human-in-the-loop** framework by prioritizing continued reductions in solvent consumption, waste generation, and energy demand (e.g., shorter columns, reduced run time, and lower organic-solvent usage where feasible) while maintaining the required analytical performance. In parallel, establishing clearer human-in-the-loop decision rules for chromatographic refinement (e.g., when to modify pH, add silanol blockers, change organic modifier, or adjust gradient steepness to address tailing/co-elution) would improve the reliability and transferability of AI-supported method development. Finally, applying the same workflow to additional pharmaceutical combinations and different HPLC platforms would further demonstrate scalability and robustness of AI-guided green method development for routine quality control.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Table 7

Comparison and assessment of Smart analytical chemistry profiles of reported and proposed [6–8] analytical techniques.

Principles of (WAC & GAC)	Reported HPLC [6]	Reported HPLC [7]	Reported HPLC [8]	Proposed Isocratic HPLC	Proposed Gradient HPLC
Red Assessment of Analytical Methods					
R1: Scope of application	80 (Pharm. only, no innovation)	80 (Pharm. only, no innovation)	90 (one component profile)	90 (AI optimization, solvent saving)	90 (AI optimization, time + solvent saving)
R2: LOD	98	100	95	90	90
R3: Precision	Validated	ICH Q2(R1)	ICH Q2(R1)	ICH Q2(R2)	ICH Q2(R2)
R4: Accuracy	Validated	ICH Q2(R1)	ICH Q2(R1)	ICH Q2(R2)	ICH Q2(R2)
Red Score (R)	89	90	92.5	90	90
Green Assessment of Analytical Methods					
AGREE tool					
Run Time (min)	11	13	17	5	6
Green Score (G)	58	54	53	64	65
Blue Assessment of Analytical Methods					
B1: Cost-efficiency	Moderate	Moderate	Moderate	low	low
B2: Time-efficiency	11 min	13 min	17 min	5 min	6 min
B3: Assessment Simplicity	< 3 steps	< 3 steps	< 3 steps	< 3 steps	< 3 steps
B4: Portability Minaturization Automation	Not portable, not miniaturized, automation	Not portable, not miniaturized, automation	Not portable, not miniaturized, automation	Not portable, not miniaturized, automation minimizes time	Not portable, not miniaturized, automation minimizes time
Blue Score	60	50	40	70	70
White Analytical Chemistry Assessment					
WAC Score (R + G + B / 3)	69	65	62	75	75
Smart Analytical Alignment	✗ Not smart-aligned	✗ Not smart-aligned	✗ Not smart-aligned	✓ smart-aligned AI-driven, aligned with GAC and WAC principles.	✓ smart-aligned AI-driven, aligned with GAC and WAC principles.

Reported HPLC methods typically excelled in precision and accuracy but fell short in eco-friendly practices and smart adaptability. In contrast, the AI-designed and optimized methods demonstrated significant improvements in several areas:

1. Applicability: reduced runtime (5–6 min versus 11–17 min) and simplified optimization processes.
2. Eco-friendliness: higher green scores driven by lower solvent consumption and faster analysis times.
3. Innovation: enhanced alignment with GAC and WAC principles through AI integration, a feature not achieved by traditional methods.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Microsoft Copilot (<https://copilot.microsoft.com>) and, for comparative scouting outputs, ChatGPT 5.2, Google Gemini, and Perplexity, to assist with language editing and with generating initial (ex-ante) method scouting

suggestions. After using these tools, the authors reviewed and edited the manuscript and take full responsibility for the accuracy and integrity of the work.

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CRediT authorship contribution statement

M. Soner Bay: Writing – original draft, Validation, Software, Investigation, Data curation, Conceptualization. **Gizem Tiris:** Writing – original draft, Validation, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Asena Ayse Genc:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. **Nevin Erk:** Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology. **Reem Hasan Obaydo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology. **Hayam M. Lotfy:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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This research is a collaboration between women scientists from Turkey, Egypt and Syria, developing sustainable drug analysis solutions for developing countries.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.greeac.2026.100323](https://doi.org/10.1016/j.greeac.2026.100323).

Data availability

No data was used for the research described in the article.

References

- J.L. Adams, B.N. Greener, A.D. Kashuba, Pharmacology of HIV integrase inhibitors, *Curr. Opin. HIV AIDS* 7 (5) (2012) 390–400, <https://doi.org/10.1097/COH.0b013e328356e91c>.
- S. Hare, S.J. Smith, M. Metifiot, A.J. Chamiec, Y. Pommier, S.H. Hughes, P. Cherepanov, Structural and functional analyses of the second-generation integrase strand transfer inhibitor dolutegravir (S/GSK1349572), *Mol. Pharmacol.* 80 (4) (2011) 565–572, <https://doi.org/10.1124/mol.111.073189>.
- M.L. Cottrell, T. Hadzic, A.D. Kashuba, Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir, *Clin. Pharmacokinet.* 52 (11) (2013) 981–994, <https://doi.org/10.1007/s40262-013-0093-2>.
- C.M. Perry, D. Faulds, Lamivudine: a review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV infection, *Drugs* 53 (4) (1997) 657–680.
- G.J. Yuen, S. Weller, G.E. Pakes, A review of the pharmacokinetics of abacavir, *Clin. Pharmacokinet.* 47 (6) (2008) 351–371, <https://doi.org/10.2165/00003088-200847060-00001>.
- R. Prava, G. Seru, V.K. Pujala, S.B. Lagu, RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir, and dolutegravir in pharmaceutical dosage forms, *World J. Pharm. Sci.* 5 (5) (2017) 168–181.
- N. Pal, A.S. Rao, P. Ravikumar, Simultaneous HPLC method development and validation for estimation of lamivudine, abacavir and dolutegravir in combined dosage form with their stability studies, *Asian J. Chem.* 28 (2) (2016) 273–276.
- A. Labidi, S. Jebali, H. Oueslati, I. Ben Hajel, M. Ben Attia, R. Ben Said, Chaotropic chromatography method for simultaneous determination of lamivudine, abacavir and dolutegravir in pharmaceutical formulations, *Chem. Afr.* 7 (2024) 2625–2634, <https://doi.org/10.1007/s42250-024-00911-8>.
- A. Serag, M.A. Hasan, E.H. Tolba, A.M. Abdelzaher, A. Abo Elmaaty, Analysis of the ternary antiretroviral therapy dolutegravir, lamivudine and abacavir using UV spectrophotometry and chemometric tools, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 264 (2022) 120334, <https://doi.org/10.1016/j.saa.2021.120334>.
- H.M. Lotfy, N. Erk, A.A. Genc, R.H. Obaydo, G. Tiris, Artificial intelligence in chromatography: greenness and performance evaluation of AI-predicted and in-lab optimized HPLC methods for simultaneous separation of amlodipine, hydrochlorothiazide, and candesartan, *Talanta Open* 11 (2025) 100473, <https://doi.org/10.1016/j.talo.2025.100473>.
- C.M. Hussain, G. Hussain, R. Keçili, Smart analytical chemistry: integrating green, sustainable, white and AI-driven approaches in modern analysis, *TrAC Trends Anal. Chem.* 172 (2025) 118295, <https://doi.org/10.1016/j.trac.2024.118295>.
- S. Armenta, S. Garrigues, M. de la Guardia, Green analytical chemistry, *TrAC Trends Anal. Chem.* 27 (6) (2008) 497–511, <https://doi.org/10.1016/j.trac.2008.05.003>.
- P.M. Nowak, R. Wietecha-Postuszny, J. Pawliszyn, White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality, *TrAC Trends Anal. Chem.* 138 (2021) 116223, <https://doi.org/10.1016/j.trac.2021.116223>.
- United States Pharmacopeial Convention, Lamivudine. United States Pharmacopeia–National Formulary (USP 39–NF 34), United States Pharmacopeial Convention, 2016, pp. 2280–2282.
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2023). ICH guideline Q2(R2) on validation of analytical procedures – Step 2b https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r2-validation-analytical-procedures-step-2b_en.pdf.
- M.I. Helmy, M.A. El Hamd, R.H. Obaydo, D. Nashed, C.K. Nessim, Bridging pharma and sustainability: green electrochemical analysis of antiparkinsonian drug in pharmaceuticals and plasma, aligned with United Nations goals via the NQS Index, *J. Electrochem. Soc.* 171 (7) (2024) 077512, <https://doi.org/10.1149/1945-7111/ad252c>.
- H.K. Chanduluru, A. Sugumaran, P. Kannaiah, R.H. Obaydo, H.M. Lotfy, Aligning drug analysis with SDGs: spectrophotometric methods for triple antihypertensive drug using propylene carbonate and statistical Dixon's and Grubb's tests, *Green. Chem. Lett. Rev.* 18 (1) (2025) 2510297, <https://doi.org/10.1080/17518253.2024.2510297>.
- H.M. Lotfy, A.A. Genc, M.S. Bay, G. Tiris, R.H. Obaydo, N. Erk, Development of eco-friendly sensitive HPLC method for determination of letrozole and assessment of validation, *Talanta Open* 11 (2025) 100425, <https://doi.org/10.1016/j.talo.2025.100425>.
- M.A. El Hamd, R.H. Obaydo, M.I. Helmy, W.A. Mahdi, S. Alshehri, M. El-Maghrabey, C.K. Nessim, Nano-scale multi-spectroscopic analysis of the anti-fibromyalgia drug pregabalin based on dihydropyridine ring formation with sustainability and whiteness evaluation, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 325 (2025) 125151, <https://doi.org/10.1016/j.saa.2024.125151>.
- P. Prajapati, B. Rana, V.S. Pulusu, S. Shah, Method operable design region for robust RP-HPLC analysis of pioglitazone hydrochloride and teneligliptin hydrobromide hydrate: incorporating hybrid principles of white analytical chemistry and design of experiments, *Futur. J. Pharm. Sci.* 9 (2023) 93, <https://doi.org/10.1186/s43094-023-00546-5>.
- P. Prajapati, A. Shahi, A. Acharya, V.S. Pulusu, S. Shah, Robust method operable design region for economical and eco-friendly chromatographic analysis of azilsartan medoxomil and cilnidipine by incorporating a hybrid approach of green analytical chemistry and analytical quality by design, *Sep. Sci. Plus* 6 (11) (2023) 2300111, <https://doi.org/10.1002/sscp.202300111>.
- A. Barseem, R.H. Obaydo, S.H. Elagamy, Eco-friendly HPLC method for simultaneous determination of pantoprazole and domperidone: comprehensive evaluation of greenness, whiteness, and blueness, *Talanta Open* (2025) 100560, <https://doi.org/10.1016/j.talo.2025.100560>.
- A. Barseem, R.H. Obaydo, S.H. Elagamy, Micelle-enhanced green spectrofluorimetric method for the determination of avanafil in dosage forms and spiked human plasma with sustainability assessment, *Talanta Open* (2025) 100526, <https://doi.org/10.1016/j.talo.2025.100526>.
- P. Prajapati, B. Rana, V.S. Pulusu, S. Shah, Simultaneous chromatographic estimation of vildagliptin and dapagliflozin using hybrid principles of white analytical chemistry and analytical quality by design, *J. AOAC Int.* 107 (1) (2024) 212–222, <https://doi.org/10.1093/jaoacint/qsad108>.
- P. Prajapati, M. Salunkhe, V.S. Pulusu, S. Shah, Integrated approach of white analytical chemistry and analytical quality by design to multipurpose RP-HPLC method for synchronous estimation of multiple fixed-dose combinations of paracetamol, *Chem. Afr.* 7 (2024) 1353–1371, <https://doi.org/10.1007/s42250-023-00819-9>.
- P. Prajapati, B. Rana, V.S. Pulusu, S. Shah, Multipurpose RP-HPLC method for simultaneous estimation of fixed-dose combinations of anti-diabetic drugs: integrating green, economical, and robust approaches with design of experiments and white analytical chemistry, *Chem. Afr.* 7 (2024) 1385–1400, <https://doi.org/10.1007/s42250-023-00835-9>.
- P. Prajapati, A. Shahi, A. Acharya, V.S. Pulusu, S. Shah, 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, *J. Chromatogr. Sci.* 62 (10) (2024) 938–952, <https://doi.org/10.1093/chromsci/bmad054>.