IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Green Analytical Chemistry In Pharmaceutical Quality Control: Sustainable RP-HPLC Method Development For Antidiabetic Fixed-Dose Combinations

Shubham Mahajan [1*], Dr. Shipra Thapar [1], Vishal [1], Sahil Mahajan [1]

[1] Department of Pharmaceutical chemistry, CT University, Ferozepur Rd, Sidhwan Khurd, Punjab 142023, India

ABSTRACT

Green Analytical Chemistry (GAC) has emerged as a critical approach to reduce the environmental impact of pharmaceutical analysis while maintaining analytical efficiency and regulatory compliance. Reversed-phase high-performance liquid chromatography (RP-HPLC), although widely used in pharmaceutical quality control, is traditionally associated with high solvent consumption, energy usage, and hazardous waste generation. This review critically evaluates the integration of GAC principles into RP-HPLC method development, with a specific focus on antidiabetic fixed-dose combinations (FDCs). The complexity of these formulations, arising from diverse physicochemical properties of combined drugs, necessitates advanced optimisation strategies to achieve efficient and sustainable analysis. Recent studies demonstrate that the use of green solvents, such as ethanol and water-rich mobile phases, combined with Analytical Quality by Design (AQbD) and Design of Experiments (DoE), can significantly reduce solvent usage and improve method robustness. Furthermore, the application of greenness assessment tools, including AGREE, GAPI, NEMI, and BAGI, enables quantitative evaluation of environmental impact. Alternative techniques, such as spectroscopic and miniaturised analytical systems, further support sustainability. Despite these advancements, challenges related to solvent compatibility, sensitivity, and regulatory acceptance persist. Overall, integrating green chemistry principles with advanced analytical strategies offers a promising approach to developing sustainable RP-HPLC methods for pharmaceutical quality control.

KEYWORDS

Green analytical chemistry; RP-HPLC; Antidiabetic drugs; Fixed-dose combinations; Analytical Quality by Design; Green solvents; Pharmaceutical quality control; Sustainability

1. INTRODUCTION

The pharmaceutical industry has historically relied on robust and highly sensitive analytical techniques to ensure the quality, safety, and efficacy of drug products. Among these, reversed-phase high-performance liquid chromatography (RP-HPLC) has remained the most widely employed analytical tool for routine quality control due to its high resolution, reproducibility, and versatility across diverse drug

classes. However, despite its analytical superiority, conventional RP-HPLC methods are increasingly being questioned due to their environmental impact, particularly the extensive use of hazardous organic solvents such as acetonitrile and methanol, high energy consumption, and significant waste generation. These concerns have necessitated a transition toward more sustainable and environmentally responsible analytical practices.

The concept of Green Analytical Chemistry (GAC) has emerged as a strategic response to these challenges, aiming to reduce the ecological footprint of analytical methodologies while maintaining analytical performance. The principles of GAC emphasise minimising solvent consumption, replacing toxic reagents with safer alternatives, reducing energy requirements, and developing efficient analytical workflows. Archana and Mani (2025) highlighted that traditional analytical practices heavily depend on non-green solvents and generate substantial hazardous waste, posing risks to both the environment and laboratory personnel. They emphasised that adopting the principles of green analytical chemistry not only improves environmental safety but also enhances operational efficiency and cost-effectiveness. Similarly, Jankech et al. (2024) reported that recent advancements in analytical chemistry have increasingly focused on greener methodologies, including solvent substitution, method miniaturisation, and energy-efficient analytical designs, while emphasising the importance of objective greenness evaluation using tools such as AGREE, GAPI, and BAGI.

The relevance of green analytical chemistry is particularly significant in pharmaceutical quality control for antidiabetic drugs, especially fixed-dose combinations (FDCs). The increasing prevalence of type 2 diabetes mellitus has led to the widespread use of combination therapies that integrate drugs with distinct physicochemical and pharmacokinetic properties. These combinations, such as metformin with sulfonylureas, DPP-4 inhibitors, or SGLT2 inhibitors, present considerable analytical challenges due to differences in polarity, ionisation behaviour, and detection wavelengths. Konari and Jacob (2015) demonstrated that conventional RP-HPLC methods for multicomponent antidiabetic formulations often require complex mobile phase systems and longer run times to achieve adequate separation, thereby increasing solvent consumption and reducing method sustainability.

Recent studies have attempted to address these limitations by incorporating green chemistry principles into chromatographic method development. For instance, Al-Tannak and Hemdan (2023) developed an eco-friendly HPLC method for the simultaneous determination of three antidiabetic drugs using response surface methodology, demonstrating that optimisation through experimental design can significantly reduce solvent usage while maintaining analytical performance. Similarly, Kalpana et al. (2025) reported a green metrics-based RP-HPLC method employing Box–Behnken design for the quantification of multiple antidiabetic drugs, achieving improved greenness scores without compromising validation parameters. These findings are further supported by Ibrahim et al. (2024), who integrated green chromatography within an Analytical Quality by Design (AQbD) framework to develop a robust and sustainable method for the simultaneous analysis of single-pill antidiabetic combinations.

Despite these advancements, a substantial proportion of analytical methods for antidiabetic drugs continue to rely on conventional solvents and practices. Haneef and Khan (2023) critically evaluated liquid chromatographic methods for canagliflozin and reported that most methods only partially satisfy green chemistry criteria, with approximately 50% compliance based on greenness assessment tools. This indicates a significant gap between current analytical practices and the desired sustainability standards. Similarly, Barzani et al. (2025) noted that although HPLC and LC-MS/MS techniques offer high sensitivity and selectivity for glibenclamide analysis, their environmental impact remains a concern, necessitating the development of greener alternatives.

In addition to chromatographic approaches, alternative green analytical techniques are being explored to enhance sustainability in pharmaceutical analysis. Spectroscopic and imaging-based methods offer significant advantages, including reduced solvent consumption and simplified sample preparation. For example, Alqahtani et al. (2025) developed a green spectrofluorimetric method for the determination of nateglinide using Box–Behnken optimisation, achieving high sensitivity and excellent greenness scores as evaluated by AGREE and BAGI metrics. Similarly, de Souza Gomes et al. (2026) demonstrated the

use of smartphone-based digital imaging as a low-cost, environmentally friendly analytical tool, further supporting the shift toward sustainable quality control practices. Cherniienko et al. (2024) also emphasised the role of FTIR spectroscopy as a green analytical technique that minimises solvent use and enables real-time monitoring of pharmaceutical processes.

Moreover, integrating green analytical chemistry with advanced technological approaches is shaping the future of pharmaceutical analysis. Bredendiek et al. (2026) highlighted the importance of evaluating analytical methods not only based on their analytical performance but also considering their environmental and economic sustainability. In parallel, recent analytical reviews have emphasised the role of artificial intelligence and machine learning in optimising chromatographic conditions, reducing the number of experimental trials, and enhancing method efficiency (Barzani et al., 2026). These innovations are expected to play a critical role in developing intelligent, sustainable, and high-throughput analytical platforms. Despite the growing body of literature on green analytical chemistry, there remains a lack of comprehensive reviews specifically focusing on the development of sustainable RP-HPLC methods for antidiabetic fixed-dose combinations. Existing studies are often limited to individual drug analyses or isolated methodological advancements, without providing an integrated perspective on green method development, optimisation strategies, and practical implementation in pharmaceutical quality control. Therefore, the present review aims to provide a comprehensive and critical evaluation of green analytical chemistry in the context of RP-HPLC method development for antidiabetic fixed-dose combinations. The review focuses on integrating green chemistry principles with chromatographic optimisation strategies, evaluating greenness metrics, and analysing recent advancements in sustainable analytical methodologies. Additionally, it highlights current challenges and future perspectives to facilitate the transition toward environmentally sustainable pharmaceutical quality control systems.

2. PRINCIPLES OF GREEN ANALYTICAL CHEMISTRY

Green Analytical Chemistry (GAC) represents a systematic, transformative approach aimed at minimising the environmental, health, and economic impacts of analytical procedures while maintaining high analytical performance. It is an extension of the broader green chemistry philosophy, specifically adapted to analytical workflows, with a focus on reducing reagent consumption, eliminating hazardous substances, minimising waste generation, and improving energy efficiency. Over the past decade, the increasing emphasis on sustainability in pharmaceutical industries has accelerated the adoption of GAC principles, particularly in chromatographic techniques such as RP-HPLC.

The foundation of GAC

The foundation of GAC is built on 12 core principles that collectively guide the development of environmentally benign analytical methods. These principles include reducing sample size and the number of analyses, minimising the use of hazardous reagents, generating less waste, preferring safer solvents, improving energy efficiency, automating, and performing real-time analysis. Archana and Mani (2025) emphasized that traditional analytical techniques often violate several of these principles due to their dependence on toxic solvents such as acetonitrile and methanol, as well as energy-intensive instrumentation, thereby contributing significantly to environmental pollution and occupational hazards. They further highlighted that adopting GAC principles not only improves environmental safety but also enhances analytical efficiency by reducing operational costs and simplifying workflows.

In the context of chromatographic analysis, particularly RP-HPLC, implementing GAC principles involves several strategic modifications. These include replacing conventional organic solvents with greener alternatives such as ethanol or water-rich mobile phases, reducing flow rates to decrease solvent consumption, shortening analysis time through optimised chromatographic conditions, and adopting miniaturised systems. Jankech et al. (2024) reported that recent advancements in liquid chromatography and capillary electrophoresis have increasingly focused on these strategies, leading to the development of methods that are not only analytically robust but also environmentally sustainable. They also emphasised that the greenness of an analytical method should not be judged qualitatively but must be quantitatively assessed using standardised evaluation tools.

Greenness assessment metrics

To address this need, several greenness assessment metrics have been developed, each providing a structured framework for evaluating the environmental impact of analytical methods. Among these, the National Environmental Methods Index (NEMI), Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), Analytical GREEnness (AGREE), and Blue Applicability Grade Index (BAGI) are the most widely used. These tools differ in their evaluation approaches but collectively provide a comprehensive assessment of method sustainability. For instance, AGREE integrates all 12 principles of GAC into a single numerical score, while GAPI provides a visual representation of environmental impact across the different stages of the analytical process. Jankech et al. (2024) critically analysed these tools and concluded that a combination of metrics offers a more accurate and holistic evaluation of method greenness.

The application of these metrics has revealed that many existing chromatographic methods still fall short of achieving optimal sustainability. Haneef and Khan (2023) evaluated liquid chromatographic methods for canagliflozin using the NEMI tool and reported that most methods met only partially the green analytical criteria, with approximately 50% of the parameters meeting sustainability requirements. This finding highlights the ongoing reliance on conventional solvents and the need for further optimisation to improve environmental performance. Similarly, Mahgoub et al. (2025) demonstrated the use of multiple greenness assessment tools, including NEMI, AGREE, and BAGI, to evaluate an RP-HPLC method for the simultaneous determination of multiple drugs, confirming that comprehensive greenness evaluation is essential for validating the sustainability of analytical methods. In addition to solvent selection and method optimisation, GAC also emphasises the importance of sustainable sample preparation techniques. Traditional sample preparation methods often involve large volumes of organic solvents and multiple processing steps, resulting in increased waste and energy consumption. Modern approaches such as microextraction, solid-phase extraction (SPE), and direct dilution techniques have been developed to address these challenges. Jankech et al. (2024) highlighted that reducing or eliminating sample preparation steps is a key strategy for enhancing method greenness, as it significantly reduces both solvent use and analysis time.

Another critical aspect of GAC is energy efficiency. Analytical instruments such as HPLC systems consume significant amounts of energy, particularly during long analytical runs and high-pressure operations. The development of faster chromatographic methods, the use of shorter columns, and the optimisation of operating conditions can substantially reduce energy consumption. Bredendiek et al. (2026) emphasised that sustainability assessment should not be limited to chemical usage alone but must also consider energy consumption and economic factors, as these collectively determine the overall environmental impact of an analytical method. The integration of green analytical chemistry with advanced methodological frameworks, such as Analytical Quality by Design (AQbD), has further advanced the development of sustainable analytical methods. AQbD provides a systematic approach for method optimisation by identifying critical method parameters and their interactions, thereby reducing the need for trial-and-error experimentation. Ibrahim et al. (2024) demonstrated that combining AQbD with green chromatography principles enables the development of robust, efficient, and environmentally friendly analytical methods for complex pharmaceutical formulations, including antidiabetic drug combinations.

Furthermore, emerging analytical technologies are advancing GAC significantly. Spectroscopic techniques such as FTIR offer solvent-free or low-solvent alternatives for pharmaceutical analysis. Cherniienko et al. (2024) highlighted that FTIR spectroscopy enables real-time monitoring of chemical processes with minimal environmental impact, making it an attractive tool for green analytical applications. Similarly, innovative approaches such as smartphone-based digital imaging have been explored for rapid, eco-friendly analysis, as demonstrated by de Souza Gomes et al. (2026), who developed a low-cost, sustainable method for quantitative analysis using digital imaging techniques. Despite these advancements, several challenges hinder the widespread adoption of GAC in pharmaceutical analysis. These include limitations in the availability of suitable green solvents for certain

analytes, potential compromises in analytical sensitivity and resolution, and the lack of standardized regulatory guidelines for green method validation. Additionally, the transition from conventional to green analytical methods requires significant investment in method development and validation, which may pose practical challenges for routine quality control laboratories. Overall, the principles of green analytical chemistry provide a comprehensive framework for developing sustainable analytical methods in pharmaceutical quality control. The integration of these principles into RP-HPLC method development, particularly for complex antidiabetic fixed-dose combinations, has significant potential to reduce environmental impact while maintaining analytical performance. However, achieving this balance requires a systematic approach that combines green chemistry principles, advanced optimization strategies, and rigorous greenness evaluation metrics

Table 1. Comparative Evaluation of Green Analytical Chemistry (GAC) Metrics

Metric	Principle/Framework	Evaluation Type	Key Features	Reported Application
NEMI (National Environmental Methods Index)	Hazard identification	Qualitative (quadrant-based)	Simple, rapid assessment; indicates presence of hazardous reagents	Used for canagliflozin LC methods (Haneef & Khan, 2023)
Analytical Eco-Scale	Penalty point system	Semi-quantitative	Assigns penalty points based on reagent toxicity, waste, energy	Applied in multiple green HPLC studies (Mahgoub et al., 2025)
GAPI (Green Analytical Procedure Index)	Entire workflow assessment	Visual (color-coded pentagram)	Evaluates sample prep, reagents, instrumentation	Reviewed extensively (Jankech et al., 2024)
AGREE (Analytical GREEnness Metric)	12 GAC principles	Quantitative (score 0–1)	Holistic evaluation integrating all green principles	Used in nateglinide analysis (Alqahtani et al., 2025)
BAGI (Blue Applicability Grade Index)	Practical sustainability	Quantitative	Combines greenness with applicability and performance	Applied in fluorescence methods (Mahgoub et al., 2025)

3. RP-HPLC IN PHARMACEUTICAL QUALITY CONTROL

Reversed-phase high-performance liquid chromatography (RP-HPLC) remains the cornerstone analytical technique in pharmaceutical quality control due to its exceptional capability to separate, identify, and quantify compounds across a wide range of physicochemical properties. Its widespread adoption is attributed to its high resolution, reproducibility, sensitivity, and compatibility with various detection systems, including UV, PDA, and mass spectrometry. In pharmaceutical industries, RP-HPLC is routinely employed for assay determination, impurity profiling, stability studies, dissolution testing, and bioanalytical applications, making it indispensable for regulatory compliance and product quality assurance.

The fundamental principle of RP-HPLC

The fundamental principle of RP-HPLC involves the partitioning of analytes between a non-polar stationary phase, typically C18 silica, and a relatively polar mobile phase composed of aqueous buffers and organic modifiers such as methanol or acetonitrile. This configuration allows for effective separation of compounds based on hydrophobic interactions, making RP-HPLC particularly suitable for analyzing complex pharmaceutical formulations. However, despite their analytical advantages, conventional RP-HPLC methods are associated with several limitations, particularly in terms of sustainability. The extensive use of organic solvents, long run times, and high energy consumption contribute significantly to environmental pollution and operational costs. The reliance on toxic organic solvents is one of the most critical challenges associated with traditional RP-HPLC methods. Acetonitrile and methanol, commonly used as mobile-phase components, pose significant environmental and health risks due to their toxicity, volatility, and disposal concerns. Lustosa and Kogawa (2024) reported that acetonitrile remains the most frequently used solvent in pharmaceutical analytical methods, despite the availability of safer alternatives such as ethanol, highlighting a persistent gap between analytical practice and green chemistry principles. Similarly, Archana and Mani (2025) emphasised that the continued use of hazardous solvents in analytical procedures contributes to increased waste generation and occupational exposure risks, necessitating the development of greener alternatives.

In addition to solvent-related concerns, the efficiency of RP-HPLC methods is often limited by long analysis times and high solvent consumption. Conventional methods may require run times exceeding 10–20 minutes per sample, leading to increased solvent usage and reduced throughput. This becomes particularly problematic in routine quality control laboratories where large numbers of samples must be analyzed efficiently. Advances in chromatographic technology, including the use of shorter columns, smaller particle sizes, and optimized flow rates, have enabled significant reductions in analysis time and solvent consumption. Al-Tannak and Hemdan (2023) demonstrated that the application of response surface methodology in HPLC method development can effectively optimize chromatographic conditions, resulting in reduced solvent usage and improved method efficiency for the simultaneous determination of antidiabetic drug combinations. The analytical complexity increases significantly when dealing with fixed-dose combinations (FDCs), particularly in the case of antidiabetic drugs. These formulations often contain compounds with markedly different physicochemical properties, including polarity, solubility, and ionization characteristics, which complicate chromatographic separation. Konari and Jacob (2015) reported that the simultaneous analysis of multicomponent antidiabetic drug combinations using RP-HPLC requires careful optimization of mobile phase composition, pH, and flow rate to achieve adequate resolution and peak symmetry. These requirements often lead to the use of complex mobile phase systems and gradient elution techniques, which can further increase solvent consumption and reduce method sustainability.

Recent developments in analytical chemistry involving RP-HPLC method development

Recent developments in analytical chemistry have focused on addressing these challenges by integrating green chemistry principles into RP-HPLC method development. Kalpana et al. (2025) reported a green metrics-based RP-HPLC method for the simultaneous quantification of multiple antidiabetic drugs using Box–Behnken design, demonstrating that experimental design approaches can effectively optimize chromatographic conditions while reducing environmental impact. Similarly, Kabil et al. (2025) developed a green HPLC method for the analysis of antidiabetic and gastric suppressant drugs, highlighting the feasibility of using eco-friendly solvents and optimized conditions to achieve sustainable analytical performance.

The application of Analytical Quality by Design (AQbD) has further enhanced the development of robust and sustainable RP-HPLC methods. AQbD provides a systematic framework for method development by identifying critical method parameters and their interactions, enabling the establishment of a method operable design region (MODR) that ensures consistent performance. Magdy et al. (2023) demonstrated the successful application of AQbD in developing and validating an HPLC method for the simultaneous estimation of multiple drugs, emphasising that this approach not only improves method robustness but

also reduces the need for extensive experimental trials, thereby minimising resource consumption. Ibrahim et al. (2024) further extended this approach by integrating green chromatographic principles into an AQbD framework, thereby developing environmentally sustainable methods for the simultaneous analysis of antidiabetic drug combinations.

Despite these advancements, significant challenges remain in achieving fully green RP-HPLC methods. One of the primary limitations is the compatibility of green solvents with chromatographic systems and analytes. While ethanol- and water-rich mobile phases are considered environmentally friendly, they may not always deliver the same chromatographic performance as conventional solvents, particularly for highly hydrophobic compounds. Haneef and Khan (2023) highlighted that many reported methods for canagliflozin still rely on acetonitrile due to its superior elution strength and compatibility with detection systems, indicating the need for further research to identify suitable green alternatives. Another important consideration is the balance between analytical performance and sustainability. High sensitivity and selectivity are essential for pharmaceutical quality control, particularly for impurity profiling and trace-level analysis. Barzani et al. (2025) emphasised that while techniques such as LC-MS/MS offer superior sensitivity for the analysis of antidiabetic drugs, their complexity and resource requirements may limit their applicability in routine quality control. Therefore, the development of green RP-HPLC methods must ensure that environmental benefits do not come at the expense of analytical reliability.

In addition to chromatographic methods, alternative analytical techniques are being explored to complement or replace RP-HPLC in certain applications. Spectrophotometric and spectrofluorimetric methods offer advantages in terms of simplicity, cost-effectiveness, and reduced solvent usage. Al-Mutairi et al. (2026) demonstrated the development of green UV spectrophotometric methods for the simultaneous determination of etoricoxib and tramadol, achieving high accuracy and precision while minimizing environmental impact. Similarly, Alqahtani et al. (2025) developed a green spectrofluorimetric method for the quantification of nateglinide, highlighting the potential of alternative analytical approaches for sustainable pharmaceutical analysis. Overall, RP-HPLC continues to play a central role in pharmaceutical quality control, particularly for the analysis of complex formulations such as antidiabetic fixed-dose combinations. However, the growing emphasis on sustainability has necessitated a shift toward greener analytical practices. The integration of green chemistry principles, advanced optimisation strategies, and comprehensive greenness evaluation metrics offers a promising pathway to develop sustainable RP-HPLC methods. Nevertheless, achieving this transition requires a careful balance between environmental considerations and analytical performance, supported by continuous innovation and regulatory acceptance.

4. GREEN SOLVENTS AND MOBILE PHASE OPTIMIZATION IN RP-HPLC

The selection of solvents and optimisation of mobile phase composition are among the most critical determinants of both analytical performance and environmental impact in RP-HPLC. Conventional chromatographic methods predominantly rely on organic solvents such as acetonitrile and methanol due to their favourable physicochemical properties, including low viscosity, high elution strength, and compatibility with UV detection systems. However, these solvents pose significant environmental and health concerns, including toxicity, flammability, and challenges in waste disposal. Consequently, the replacement of hazardous solvents with greener alternatives has become a central focus in the development of sustainable RP-HPLC methods.

Green solvent

Green solvent selection is guided by criteria such as low toxicity, biodegradability, renewability, and minimal environmental persistence. Among the available alternatives, ethanol has emerged as one of the most promising green solvents for chromatographic applications. It is less toxic, derived from renewable sources, and exhibits suitable polarity for many pharmaceutical compounds. Lustosa and Kogawa (2024) highlighted that although many drugs are readily soluble in ethanol, analytical methods continue to favour acetonitrile, indicating a significant gap between solvent availability and actual analytical practice. This observation underscores the need for a systematic redesign of methods to facilitate the transition toward greener solvent systems. Water, being the most environmentally benign solvent, plays a crucial role in

green chromatography. The development of high-aqueous mobile-phase systems, often referred to as "water-rich RP-HPLC," has attracted increasing attention. These systems aim to reduce or eliminate the use of organic solvents while maintaining adequate chromatographic performance. However, challenges such as poor retention of hydrophilic compounds, phase collapse of stationary phases, and limited solubility of certain analytes must be addressed. Despite these limitations, advancements in stationary phase technology and method optimisation strategies have enabled the successful application of water-rich systems in pharmaceutical analysis.

Green solvents for antidiabetic fixed-dose combinations

The complexity of antidiabetic fixed-dose combinations further complicates solvent selection and mobile phase optimisation. These formulations often contain drugs with diverse physicochemical properties, ranging from highly polar compounds such as metformin to relatively lipophilic agents such as SGLT2 inhibitors. Achieving efficient separation under green conditions requires careful adjustment of mobile phase composition, pH, and buffer strength. Al-Tannak and Hemdan (2023) demonstrated that the use of response surface methodology allows for systematic optimisation of mobile phase parameters, enabling the development of eco-friendly HPLC methods for multi-component antidiabetic formulations. Their study highlighted that reducing organic solvent content while optimising pH and flow rate can achieve adequate resolution without compromising method performance.

Experimental design approaches such as Box–Behnken and central composite design have become valuable tools for optimising chromatographic conditions in a sustainable manner. Kalpana et al. (2025) employed a Box–Behnken design to develop a green RP-HPLC method for the quantification of multiple antidiabetic drugs, demonstrating that statistical optimisation can significantly reduce solvent consumption and analysis time while maintaining analytical robustness. Similarly, Ibrahim et al. (2024) integrated green solvent selection into an Analytical Quality by Design (AQbD) framework, enabling the identification of critical method parameters and their interactions, ultimately leading to the establishment of a method-operable design region (MODR) that ensures both robustness and sustainability. In addition to conventional green solvents, emerging solvent systems such as ionic liquids and deep eutectic solvents (DES) are being explored as potential alternatives in chromatographic analysis. These solvents offer unique physicochemical properties, including tunable polarity and low volatility, making them attractive for green analytical applications. However, their widespread adoption is currently limited by factors such as high cost, limited availability, and incomplete understanding of their environmental impact. Nevertheless, ongoing research in this area is expected to expand their applicability in pharmaceutical analysis.

Optimising mobile phase pH is another critical factor influencing chromatographic separation, particularly for ionizable compounds such as antidiabetic drugs. The ionisation state of analytes affects their retention behaviour, peak shape, and resolution. For instance, metformin, being highly polar and basic, exhibits poor retention in conventional RP-HPLC systems unless appropriate pH adjustments are made. In contrast, lipophilic drugs such as canagliflozin require stronger organic modifiers for effective elution. Haneef and Khan (2023) reported that many chromatographic methods for canagliflozin rely on acetonitrile-based mobile phases due to its superior elution strength, highlighting the challenge of balancing chromatographic efficiency with environmental sustainability.

Buffer selection also plays a significant role in mobile phase optimisation. While phosphate buffers are commonly used due to their excellent buffering capacity and compatibility with chromatographic systems, they raise environmental concerns about waste disposal and potential interference with mass spectrometric detection. The use of volatile buffers such as ammonium acetate or ammonium formate offers a greener alternative, particularly in LC-MS applications. However, their buffering capacity and stability must be carefully considered during method development. Another important strategy for enhancing the sustainability of RP-HPLC methods is reducing solvent consumption by optimising flow rate and run time. Shorter analysis times not only reduce solvent usage but also improve sample throughput and energy efficiency. Mahgoub et al. (2025) demonstrated that optimised RP-HPLC methods with reduced run times can achieve high analytical performance while minimising environmental impact.

Their study also highlighted the importance of evaluating method greenness using multiple assessment tools, confirming that solvent reduction is a key contributor to overall sustainability.

Temperature optimization is an additional parameter that can influence both chromatographic performance and sustainability. Elevated column temperatures can reduce solvent viscosity, improve mass transfer, and enhance separation efficiency, thereby allowing for lower flow rates and reduced solvent consumption. However, the energy requirements associated with temperature control must be considered when evaluating overall method sustainability. Bredendiek et al. (2026) emphasised that a comprehensive assessment of analytical sustainability should include not only chemical usage but also energy consumption and economic factors.

Despite progress in green solvent selection and mobile-phase optimisation, several challenges remain. The substitution of conventional solvents with greener alternatives may alter chromatographic behaviour, including reduced resolution or altered retention times, necessitating extensive method revalidation. Additionally, regulatory acceptance of green analytical methods is still evolving, with limited guidelines for evaluating and approving them. These challenges highlight the need for continued research and standardisation to facilitate the adoption of green chromatography in routine pharmaceutical analysis.

Overall, the transition toward green solvents and optimised mobile-phase conditions represents a critical step toward sustainable RP-HPLC methods for pharmaceutical quality control. By integrating green solvent selection, statistical optimisation techniques, and comprehensive greenness evaluation, it is possible to develop analytical methods that are both environmentally friendly and analytically robust. This approach is particularly important for the analysis of complex antidiabetic fixed-dose combinations, where achieving efficient separation under sustainable conditions remains a key challenge.

Table 2. Green vs Conventional Solvents in RP-HPLC for Pharmaceutical Analysis

Solvent	**Toxicity Level**	**Environmental Impact**	**Chromatographic Performance**	**Application in Studies**
Acetonitrile	High	Hazardous waste, toxic	Excellent elution strength, low viscosity	Widely used in conventional methods (Lustosa & Kogawa, 2024; Haneef & Khan, 2023)
Methanol	Moderate	Toxic, flammable	Good performance, higher viscosity than ACN	Used in many traditional RP-HPLC methods
Ethanol	Low	Biodegradable, renewable	Moderate elution strength; greener alternative	Recommended in green HPLC methods (Kalpana et al., 2025)
Water (aqueous systems)	Minimal	Environmentally benign	Limited elution strength for hydrophobic drugs	Used in water-rich chromatography
Supercritical CO_2	Very low	Green, recyclable	High efficiency in SFC systems	Applied in impurity analysis (Jambo et al., 2024)

5. ANALYTICAL QUALITY BY DESIGN (AQbD) IN GREEN RP-HPLC METHOD DEVELOPMENT

The development of sustainable, robust analytical methods requires a systematic, scientifically driven approach that goes beyond conventional trial-and-error experimentation. Analytical Quality by Design (AQbD) has emerged as a powerful framework for method development, enabling the identification, understanding, and control of critical variables that influence analytical performance. When integrated with Green Analytical Chemistry (GAC) principles, AQbD provides a structured pathway for designing

RP-HPLC methods that are not only analytically reliable but also environmentally sustainable. AQbD is rooted in the concept of predefined objectives, commonly referred to as the Analytical Target Profile (ATP), which defines the intended purpose of the analytical method in terms of accuracy, precision, sensitivity, and robustness. In the context of green RP-HPLC, ATP is expanded to include sustainability parameters such as reduced solvent consumption, minimal energy use, and lower environmental impact. Ibrahim et al. (2024) demonstrated that incorporating green chemistry considerations into the ATP enables the development of analytical methods that achieve both performance and sustainability goals, particularly for the simultaneous analysis of antidiabetic drug combinations.

A critical component of AQbD is the identification of Critical Quality Attributes (CQAs), which represent the measurable characteristics of the analytical method that must be controlled to ensure acceptable performance. In RP-HPLC, CQAs typically include parameters such as resolution, retention time, peak symmetry, and sensitivity. These attributes are influenced by various method variables, known as Critical Method Parameters (CMPs), including mobile phase composition, pH, flow rate, column temperature, and detection wavelength. The systematic evaluation of these parameters is essential for understanding their impact on method performance and identifying optimal operating conditions.

Design of Experiments (DoE) in AQbD

Design of Experiments (DoE) plays a central role in AQbD by enabling the simultaneous evaluation of multiple factors and their interactions. Unlike traditional one-factor-at-a-time approaches, DoE provides a comprehensive understanding of the analytical system, allowing for efficient optimisation with fewer experimental runs. Al-Tannak and Hemdan (2023) utilised response surface methodology to develop an eco-friendly HPLC method for the simultaneous determination of multiple antidiabetic drugs, demonstrating that DoE can effectively reduce solvent consumption and analysis time while maintaining analytical accuracy. Similarly, Kalpana et al. (2025) employed a Box–Behnken design to optimise chromatographic conditions for the quantification of antidiabetic drugs, achieving improved greenness metrics alongside robust analytical performance. The integration of DoE within AQbD facilitates the establishment of a Method Operable Design Region (MODR), which defines the multidimensional space within which the method consistently meets predefined performance criteria. The MODR represents a robust operating window that accommodates minor variations in method parameters without compromising analytical quality. This is particularly important in green RP-HPLC, where the use of alternative solvents and reduced resource consumption may introduce variability in chromatographic behaviour. Magdy et al. (2023) demonstrated that applying AQbD to HPLC method development enables the identification of optimal conditions that ensure consistent performance across different experimental settings, thereby enhancing method reliability and reproducibility.

One of the key advantages of AQbD in the context of green analytical chemistry is its ability to minimise resource consumption during method development. By systematically exploring the design space and identifying optimal conditions early in the development process, AQbD reduces the number of experimental trials required, thereby conserving solvents, reagents, and energy. This aligns directly with the principles of GAC, which emphasise waste reduction and efficient resource utilisation. Ibrahim et al. (2024) further highlighted that AQbD-driven green chromatography not only improves method robustness but also contributes to environmental sustainability by reducing the overall analytical footprint. In addition to optimising chromatographic conditions, AQbD also supports integrating greenness assessment metrics into the method development process. By evaluating the environmental impact of different experimental conditions within the design space, it is possible to identify optimal conditions that balance analytical performance with sustainability. Mahgoub et al. (2025) demonstrated the application of multiple greenness evaluation tools, including NEMI, AGREE, and BAGI, in assessing an RP-HPLC method, emphasising the importance of comprehensive greenness evaluation in method validation. The incorporation of such metrics within the AQbD framework enables the development of analytical methods that are quantitatively optimized for both performance and environmental impact.

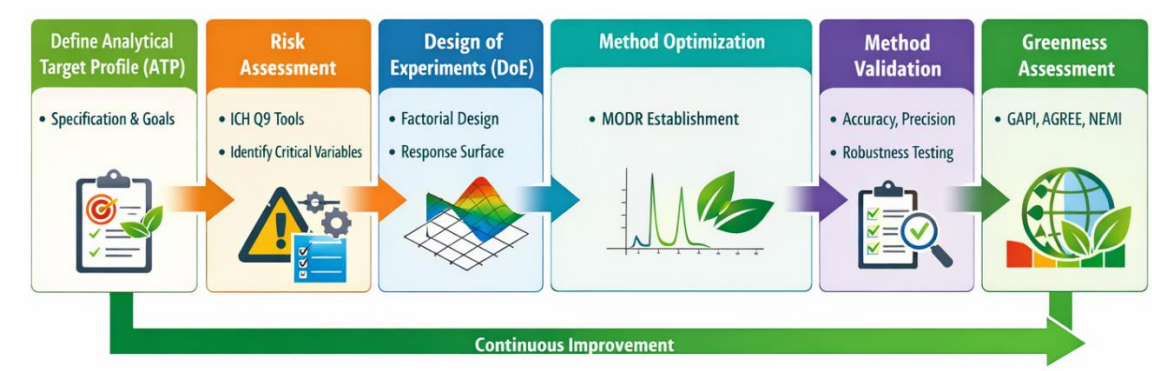

Figure 1. Analytical QbD workflow for green RP-HPLC method development

The application of AQbD in the analysis of antidiabetic fixed-dose combinations

The application of AQbD is particularly beneficial for the analysis of antidiabetic fixed-dose combinations, which pose significant analytical challenges due to multiple active pharmaceutical ingredients with diverse physicochemical properties. The simultaneous optimisation of multiple chromatographic parameters is essential to achieve adequate separation and accurate quantification of these components. Bhatia et al. (2026) demonstrated the use of a DoE-based RP-HPLC method for the estimation of metformin and glimepiride in combined dosage forms, highlighting the effectiveness of AQbD in addressing the complexities of multicomponent analysis. Furthermore, AQbD facilitates regulatory compliance by providing a comprehensive understanding of method performance and variability. Regulatory guidelines such as ICH Q2(R1) emphasise the importance of method validation parameters, including specificity, accuracy, precision, linearity, and robustness. The systematic approach of AQbD ensures that these parameters are thoroughly evaluated and controlled, thereby enhancing the reliability of the analytical method. This is particularly important for green analytical methods, where deviations from conventional practices may require additional validation to demonstrate equivalence or superiority. Despite its advantages, the implementation of AQbD in green RP-HPLC method development is not without challenges. The application of DoE requires statistical expertise and specialized software, which may not be readily available in all laboratories. Additionally, the selection of appropriate experimental designs and the interpretation of complex data sets can be time-consuming. However, the long-term benefits of AQbD, including improved method robustness, reduced resource consumption, and enhanced sustainability, outweigh these initial challenges.

Emerging trends in analytical science are further enhancing the capabilities of AQbD through the integration of artificial intelligence (AI) and machine learning (ML) techniques. These technologies enable the analysis of large data sets, prediction of optimal chromatographic conditions, and automation of method development processes. Barzani et al. (2026) highlighted that AI-driven analytical approaches have the potential to significantly improve method optimization, reduce experimental workload, and enhance the precision of analytical results, thereby supporting the development of intelligent and sustainable analytical systems. In conclusion, the integration of AQbD with green analytical chemistry principles represents a powerful strategy for developing sustainable RP-HPLC methods for pharmaceutical quality control. By providing a systematic framework for method optimization, resource conservation, and performance evaluation, AQbD enables the development of analytical methods that meet both regulatory and environmental requirements. This approach is particularly valuable for the analysis of complex antidiabetic fixed-dose combinations, where achieving a balance between analytical performance and sustainability remains a critical challenge.

6. APPLICATION OF GREEN RP-HPLC IN ANTIDIABETIC FIXED-DOSE COMBINATIONS

The application of green RP-HPLC methodologies in the analysis of antidiabetic fixed-dose combinations (FDCs) represents a critical intersection between analytical performance and environmental sustainability. The growing prevalence of type 2 diabetes mellitus has led to an increased reliance on combination therapies that target multiple metabolic pathways simultaneously. These combinations, typically involving biguanides, sulfonylureas, DPP-4 inhibitors, and SGLT2 inhibitors,

present substantial analytical challenges due to their diverse physicochemical properties, which necessitate highly optimized chromatographic conditions. Consequently, the development of sustainable RP-HPLC methods for such formulations has become an area of significant research interest. One of the primary analytical challenges associated with antidiabetic FDCs is the wide polarity range of the constituent drugs. For instance, metformin is highly polar and hydrophilic, whereas drugs such as glimepiride and canagliflozin exhibit greater lipophilicity. This disparity complicates chromatographic separation, often requiring gradient elution systems and high concentrations of organic solvents to achieve adequate resolution. Konari and Jacob (2015) reported that conventional RP-HPLC methods for multicomponent antidiabetic formulations frequently involve complex mobile phase compositions and extended run times, leading to increased solvent consumption and reduced sustainability. These findings highlight the need for innovative strategies to achieve efficient separation under green conditions.

Recent advancements have demonstrated that the integration of green chemistry principles with systematic optimization approaches can effectively address these challenges. Al-Tannak and Hemdan (2023) developed an eco-friendly HPLC method for the simultaneous determination of three antidiabetic drugs using response surface design. Their study demonstrated that careful optimization of mobile phase composition, pH, and flow rate can significantly reduce solvent usage while maintaining acceptable resolution and sensitivity. Similarly, Kalpana et al. (2025) reported a green metrics-based RP-HPLC method employing Box–Behnken design for the simultaneous quantification of multiple antidiabetic drugs, achieving improved greenness scores alongside robust analytical performance.

The incorporation of Analytical Quality by Design (AQbD) has further enhanced the development of green RP-HPLC methods for antidiabetic FDCs. Ibrahim et al. (2024) demonstrated that integrating AQbD with green chromatography principles allows for the systematic identification of critical method parameters and their interactions, enabling the development of robust and sustainable methods for single-pill antidiabetic combinations. This approach facilitates the establishment of a method operable design region (MODR), ensuring consistent performance while minimizing resource consumption. Magdy et al. (2023) also emphasized the effectiveness of AQbD in optimizing HPLC methods for multi-component drug analysis, highlighting its role in reducing experimental trials and enhancing method robustness. The greenness of analytical methods for antidiabetic drugs has been evaluated using various assessment tools, revealing significant opportunities for improvement. Haneef and Khan (2023) critically reviewed liquid chromatographic methods for canagliflozin and reported that most methods achieve only partial compliance with green analytical chemistry principles, with approximately 50% greenness based on NEMI evaluation. This indicates that despite advancements in method development, there is still a considerable reliance on conventional solvents and practices. Similarly, Barzani et al. (2025) highlighted that while HPLC and LC-MS/MS methods provide high sensitivity and specificity for the analysis of glibenclamide, their environmental impact remains a concern, necessitating the development of greener alternatives.

In response to these challenges, several studies have explored the use of eco-friendly solvents and optimized chromatographic conditions to enhance method sustainability. Kabil et al. (2025) developed a green HPLC method for the analysis of antidiabetic and gastric suppressant drugs, demonstrating that the use of environmentally benign solvents and optimized conditions can achieve reliable analytical performance while reducing environmental impact. Bhatia et al. (2026) further demonstrated the application of design of experiments (DoE) in developing an RP-HPLC method for the simultaneous estimation of metformin and glimepiride, highlighting the effectiveness of statistical optimization in addressing the complexities of multicomponent analysis. In addition to chromatographic optimization, alternative analytical approaches are being explored to complement green RP-HPLC methods. Spectroscopic techniques, such as UV and fluorescence spectroscopy, offer advantages in terms of reduced solvent consumption and simplified sample preparation. Alqahtani et al. (2025) developed a green spectrofluorimetric method for the determination of nateglinide, achieving high sensitivity and excellent greenness scores using AGREE and BAGI metrics. Similarly, Al-Mutairi et al. (2026) reported green UV spectrophotometric methods for the simultaneous determination of etoricoxib and tramadol,

demonstrating that spectroscopic techniques can serve as cost-effective and environmentally friendly alternatives for routine quality control . While these methods may not fully replace chromatographic techniques, they can significantly reduce the analytical burden and contribute to overall sustainability.

The integration of advanced technologies is also playing a crucial role in enhancing the sustainability of analytical methods for antidiabetic FDCs. The use of miniaturized systems, such as micro-HPLC and capillary electrophoresis, has been shown to reduce solvent consumption and energy requirements. Jankech et al. (2024) highlighted that miniaturization and automation are key strategies for improving the greenness of analytical methods, enabling faster analysis and reduced resource usage. Furthermore, the application of artificial intelligence and machine learning in method optimization has the potential to significantly reduce experimental workload and improve analytical efficiency. Barzani et al. (2026) emphasized that AI-driven analytical approaches can enhance chromatographic optimization, enabling the development of intelligent and sustainable analytical systems. Despite these advancements, several challenges remain in the implementation of green RP-HPLC methods for antidiabetic FDCs. The substitution of conventional solvents with greener alternatives may affect chromatographic performance, including resolution, retention time, and peak symmetry, necessitating extensive method optimization and validation. Additionally, regulatory acceptance of green analytical methods is still evolving, with limited guidelines available for evaluating and approving such methods. These challenges highlight the need for continued research and collaboration between academia, industry, and regulatory bodies to facilitate the adoption of sustainable analytical practices.

Another important consideration is the balance between analytical performance and sustainability. Pharmaceutical quality control requires highly sensitive and reliable methods to ensure product safety and efficacy. Therefore, green analytical methods must be carefully designed to maintain analytical accuracy and precision while minimising environmental impact. Mahgoub et al. (2025) demonstrated that this balance can be achieved by integrating green chemistry principles with robust method validation and comprehensive greenness evaluation, thereby confirming the feasibility of sustainable analytical approaches in pharmaceutical analysis. Overall, the application of green RP-HPLC methods in the analysis of antidiabetic fixed-dose combinations represents a promising approach for achieving sustainable pharmaceutical quality control. By integrating green chemistry principles, advanced optimisation strategies, and comprehensive greenness evaluation metrics, it is possible to develop analytical methods that are both environmentally friendly and analytically robust. However, achieving widespread adoption of these methods requires continued innovation, regulatory support, and a commitment to sustainability across the pharmaceutical industry.

Table 3. Reported Green RP-HPLC Methods for Antidiabetic Drugs and Fixed-Dose Combinations

Study	Drug(s) Analyzed	Method Approach	Green Strategy Applied	Key Outcome
Al-Tannak & Hemdan (2023)	Three antidiabetic drugs	RP-HPLC with DoE	Response surface optimization, reduced solvent	Efficient separation with lower solvent consumption
Kalpana et al. (2025)	Multiple antidiabetic drugs	Box–Behnken optimized RP-HPLC	Green metrics-based optimization	Improved greenness score with robust validation
Ibrahim et al. (2024)	Antidiabetic FDCs	AQbD-based RP-HPLC	Integration of green chromatography + DoE	Robust, sustainable method with MODR establishment
Bhatia et al. (2026)	Metformin + Glimepiride	DoE-based RP-HPLC	Optimization of mobile phase and conditions	Accurate simultaneous estimation with reduced trials

Kabil et al. (2025)	Antidiabetic + gastric drugs	Green RP-HPLC	Eco-friendly solvent system	Sustainable and validated analytical method
Haneef & Khan (2023)	Canagliflozin	LC methods review	Greenness evaluation (NEMI ~50%)	Highlighted need for greener solvent replacement
Barzani et al. (2025)	Glibenclamide	Analytical review	Emphasis on green analytical integration	Identified sustainability gaps in current methods

7. GREENER SAMPLE PREPARATION TECHNIQUES IN PHARMACEUTICAL ANALYSIS

Sample preparation constitutes a critical step in pharmaceutical analysis, significantly influencing both analytical accuracy and environmental impact. Traditional sample preparation methods in RP-HPLC, such as liquid–liquid extraction (LLE) and protein precipitation, often involve large volumes of organic solvents, multiple processing steps, and extended preparation times. These practices contribute substantially to solvent consumption, waste generation, and overall environmental burden. Consequently, the development of greener sample preparation techniques has become an essential component of sustainable analytical chemistry, particularly in the context of pharmaceutical quality control of antidiabetic fixed-dose combinations. One of the fundamental strategies in green sample preparation is the reduction or elimination of organic solvent usage. Conventional extraction techniques frequently rely on toxic solvents such as chloroform, dichloromethane, and acetonitrile, which pose environmental and health hazards. In contrast, modern approaches emphasize the use of safer alternatives, including water, ethanol, and solvent-free techniques. Jankech et al. (2024) highlighted that minimizing solvent consumption during sample preparation is a key principle of Green Analytical Chemistry (GAC), and recent advancements have focused on reducing the number of preparation steps and adopting miniaturized extraction techniques to achieve this goal.

Solid-phase extraction (SPE)

Solid-phase extraction (SPE) has emerged as a widely adopted green alternative to traditional liquid–liquid extraction. SPE offers several advantages, including reduced solvent consumption, improved selectivity, and enhanced reproducibility. It enables efficient cleanup and concentration of analytes from complex matrices while minimizing waste generation. Haneef and Khan (2023) reported that SPE is increasingly used for the analysis of antidiabetic drugs such as canagliflozin, particularly in biological samples, due to its ability to reduce matrix interference and enhance method sensitivity. However, they also noted that conventional SPE methods still require optimisation to further reduce solvent usage and enhance sustainability.

Miniaturised sample preparation techniques

Miniaturised sample preparation techniques represent another significant advancement in green analytical chemistry. These techniques, including microextraction methods such as dispersive liquid–liquid microextraction (DLLME) and solid-phase microextraction (SPME), utilize significantly smaller volumes of solvents and samples, thereby reducing waste and improving efficiency. Jankech et al. (2024) emphasized that miniaturization not only reduces environmental impact but also enhances analytical throughput and sensitivity, making it particularly suitable for high-throughput pharmaceutical analysis.

Direct sample preparation approaches

Direct sample preparation approaches, such as simple dilution and filtration, have also gained attention as green alternatives, especially for pharmaceutical dosage forms where matrix complexity is relatively low. These methods eliminate the need for extensive extraction procedures, thereby reducing solvent consumption and analysis time. In the context of antidiabetic fixed-dose combinations, direct dilution techniques can be effectively applied for tablet formulations, provided that the drugs are sufficiently soluble and stable in the selected solvent system. This approach aligns well with the principles of GAC by simplifying analytical workflows and minimizing resource utilization. Automation and integration of

sample preparation with analytical instrumentation further contribute to the greening of pharmaceutical analysis. Automated systems reduce human intervention, minimize errors, and improve reproducibility while optimizing resource utilization. Additionally, the integration of sample preparation with analytical techniques, such as online SPE coupled with HPLC, allows for continuous analysis with minimal solvent usage and waste generation. These advancements are particularly beneficial in routine quality control laboratories, where large numbers of samples must be processed efficiently.

Emerging technologies in enhancing the sustainability of sample preparation

Emerging technologies are also playing a crucial role in enhancing the sustainability of sample preparation. Techniques such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) offer environmentally friendly alternatives by using non-toxic solvents such as carbon dioxide under controlled conditions. Jambo et al. (2024) demonstrated the application of supercritical fluid chromatography-mass spectrometry (SFC-MS) for the analysis of pharmaceutical impurities, highlighting its potential as a green analytical tool due to reduced solvent usage and high efficiency. Although primarily used for separation, the principles of supercritical fluid technology can be extended to sample preparation, offering a promising direction for future research. In addition to solvent reduction, simplifying analytical workflows is a key aspect of green sample preparation. Techniques that combine multiple analytical steps into a single procedure can significantly reduce resource consumption and waste generation. Cherniienko et al. (2024) highlighted that spectroscopic techniques such as FTIR can be used for direct analysis without extensive sample preparation, thereby minimising environmental impact. Similarly, de Souza Gomes et al. (2026) demonstrated that smartphone-based analytical methods can eliminate the need for complex sample preparation, offering a rapid and eco-friendly alternative for certain applications.

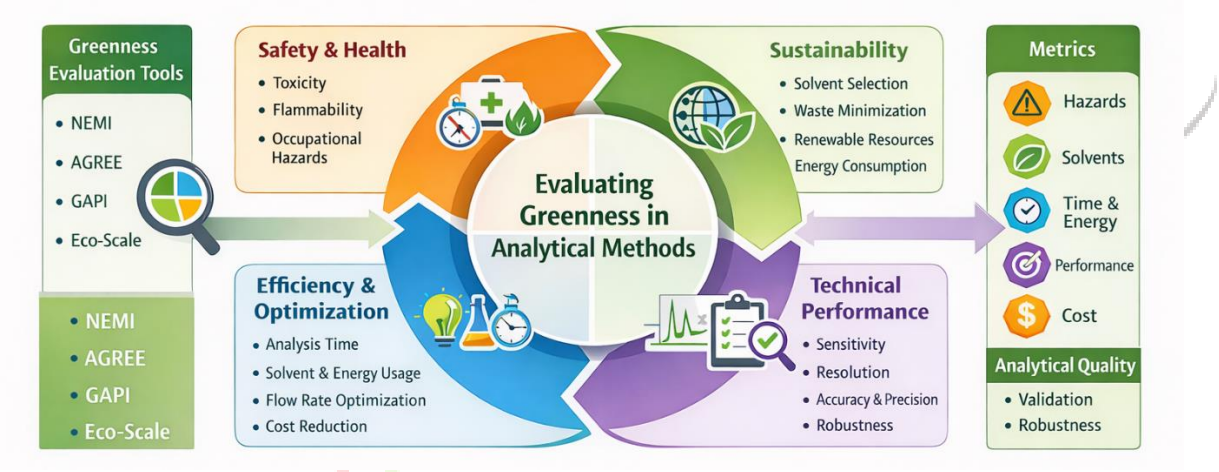

Figure 2. Comprehensive framework for evaluating greenness in analytical methods

Despite these advancements, several challenges remain in implementing green sample preparation techniques. The complexity of pharmaceutical matrices, particularly in biological samples, often necessitates selective, sensitive extraction methods that may require the use of organic solvents. Additionally, adopting new techniques may require significant investment in equipment and training, which can be a barrier for routine quality control laboratories. Furthermore, regulatory guidelines for validating green sample preparation methods are still evolving, creating uncertainty in their widespread adoption. Another important consideration is the balance between method simplicity and analytical performance. While direct and simplified sample preparation techniques offer significant environmental benefits, they must be carefully evaluated to ensure that they do not compromise analytical accuracy, precision, or sensitivity. Mahgoub et al. (2025) emphasized that the validation of green analytical methods must adhere to established regulatory standards to ensure their reliability and applicability in pharmaceutical quality control.

Overall, greener sample preparation techniques play a vital role in achieving sustainable pharmaceutical analysis. By reducing solvent consumption, simplifying workflows, and adopting innovative extraction technologies, it is possible to significantly minimize the environmental impact of analytical procedures.

In the context of antidiabetic fixed-dose combinations, the integration of green sample preparation with optimized RP-HPLC methods offers a comprehensive approach to sustainable quality control. Continued research and technological advancements are expected to further enhance the efficiency and applicability of these techniques, paving the way for greener and more sustainable pharmaceutical analysis.

8. CHALLENGES AND LIMITATIONS IN GREEN RP-HPLC FOR PHARMACEUTICAL ANALYSIS

Despite significant progress in developing green analytical methodologies, the implementation of sustainable RP-HPLC techniques in pharmaceutical quality control remains constrained by several scientific, technical, and regulatory challenges. While the principles of Green Analytical Chemistry (GAC) provide a clear framework for minimising environmental impact, their practical application often requires careful balancing between analytical performance and sustainability. One of the primary challenges lies in replacing conventional organic solvents with greener alternatives. Solvents such as acetonitrile and methanol continue to dominate RP-HPLC methods due to their favourable physicochemical properties, including low viscosity, high elution strength, and compatibility with a wide range of analytes. Although greener solvents such as ethanol and water-rich systems are increasingly being explored, they may not always provide equivalent chromatographic performance. Lustosa and Kogawa (2024) reported that despite the availability of ethanol as a safer alternative, acetonitrile remains the preferred solvent in a substantial proportion of pharmaceutical analytical methods, highlighting a gap between sustainability goals and practical implementation. Similarly, Haneef and Khan (2023) observed that many chromatographic methods for canagliflozin still rely on conventional solvents due to limitations in achieving optimal resolution and sensitivity with greener alternatives.

Another critical limitation concerns the analytical complexity of multicomponent drug formulations, particularly antidiabetic fixed-dose combinations. These formulations often contain drugs with widely varying polarity, solubility, and ionization characteristics, making it challenging to develop a single chromatographic method that is both efficient and environmentally sustainable. Konari and Jacob (2015) demonstrated that achieving adequate separation in multicomponent systems often requires complex mobile-phase compositions and gradient elution, which can increase solvent consumption and reduce method greenness. Although advanced optimisation strategies such as Design of Experiments (DoE) and Analytical Quality by Design (AQbD) have been successfully applied to address these challenges, their implementation requires specialised expertise and resources.

Sensitivity and detection limits also present significant challenges in green analytical method development. Pharmaceutical quality control often demands the detection and quantification of impurities and degradation products at trace levels. Techniques such as LC-MS/MS provide the required sensitivity but involve high operational complexity, increased energy consumption, and high costs. Barzani et al. (2025) emphasised that while high-performance chromatographic techniques offer superior analytical performance, their environmental and economic impacts must be carefully considered. In contrast, greener alternatives such as spectrophotometric and spectrofluorimetric methods may offer reduced environmental impact but may not always achieve the required sensitivity for certain applications. The integration of greenness assessment metrics into method development and validation also presents challenges. Although tools such as NEMI, GAPI, AGREE, and BAGI provide structured frameworks for evaluating method sustainability, their application is not yet standardised across the pharmaceutical industry. Jankech et al. (2024) highlighted that different metrics may yield varying assessments of method greenness, making it difficult to establish universally accepted criteria for sustainable analytical methods. This lack of standardisation complicates the comparison of different methods and may hinder regulatory acceptance.

Energy consumption is another often overlooked aspect of analytical sustainability. RP-HPLC systems require significant energy for pump operation, column temperature control, and detection systems. While efforts have been made to reduce analysis time and optimize operating conditions, the overall energy footprint of chromatographic methods remains substantial. Bredendiek et al. (2026) emphasized that a comprehensive evaluation of analytical sustainability must consider not only chemical usage but also

energy consumption and economic factors. However, the quantification and standardization of energy-related metrics in analytical chemistry remain underdeveloped. Sample preparation represents an additional area of concern in the implementation of green analytical methods. Although advances in miniaturized and solvent-free extraction techniques have reduced environmental impact, many pharmaceutical analyses still require complex sample preparation procedures involving organic solvents. Haneef and Khan (2023) noted that liquid–liquid extraction remains a commonly used technique in the analysis of antidiabetic drugs, despite its high solvent consumption and environmental burden. The adoption of greener alternatives such as solid-phase extraction and microextraction techniques is increasing but requires further optimization and validation.

Regulatory acceptance is another critical barrier to the widespread adoption of green analytical methods. Current regulatory guidelines, such as those provided by the International Council for Harmonisation (ICH), primarily focus on method validation parameters including accuracy, precision, specificity, and robustness, with limited emphasis on environmental sustainability. As a result, pharmaceutical companies may be hesitant to adopt green analytical methods that deviate from established practices without clear regulatory guidance. This highlights the need for the development of standardized guidelines that incorporate green chemistry principles into analytical method validation and approval processes. Economic considerations also play a significant role in the adoption of green analytical methods. While green methods may reduce long-term costs associated with solvent procurement and waste disposal, the initial investment required for method development, validation, and implementation can be substantial. Additionally, the transition to new analytical techniques may require changes in laboratory infrastructure and personnel training, which can pose practical challenges for routine quality control laboratories. Another limitation is the lack of awareness and training in green analytical chemistry among analytical scientists. The successful implementation of GAC principles requires a thorough understanding of both analytical techniques and sustainability concepts. Archana and Mani (2025) emphasized that increasing awareness and education in green chemistry is essential for promoting the adoption of sustainable analytical practices. Without adequate training and knowledge, the implementation of green methods may be limited.

Finally, the scalability and industrial applicability of green analytical methods remain areas of concern. While many green methods have been successfully demonstrated at the laboratory scale, their translation to large-scale industrial applications requires further validation and optimization. Factors such as method robustness, reproducibility, and compatibility with existing analytical systems must be carefully evaluated to ensure successful implementation in routine quality control environments. In summary, while green RP-HPLC methods offer significant advantages in terms of environmental sustainability and resource efficiency, their widespread adoption is hindered by several challenges related to solvent selection, analytical complexity, sensitivity, regulatory acceptance, and economic feasibility. Addressing these challenges requires a multidisciplinary approach that integrates advances in analytical chemistry, process optimization, regulatory frameworks, and education. Continued research and collaboration among academia, industry, and regulatory bodies are essential to overcome these limitations and facilitate the transition toward sustainable pharmaceutical quality control practices.

9. FUTURE PERSPECTIVES AND EMERGING TRENDS IN GREEN ANALYTICAL CHEMISTRY

The evolving landscape of pharmaceutical analysis is increasingly being shaped by the need for sustainability, efficiency, and technological advancement. Green Analytical Chemistry (GAC), when integrated with modern analytical innovations, is poised to redefine pharmaceutical quality control, particularly in the context of RP-HPLC method development for antidiabetic fixed-dose combinations. Future trends indicate a strong shift toward intelligent, miniaturised, and environmentally optimised analytical systems that can deliver high analytical performance with minimal ecological impact. One of the most promising developments in this field is the integration of artificial intelligence (AI) and machine learning (ML) into analytical method development. These technologies have the potential to revolutionise

chromatographic optimisation by predicting optimal conditions, reducing experimental trials, and enhancing method robustness. Barzani et al. (2026) highlighted that AI-driven analytical systems can significantly improve chromatographic parameter optimisation, enabling faster method development and improved reproducibility. The application of predictive modelling can assist in selecting green solvents, optimising mobile phase composition, and identifying critical method parameters, thereby aligning analytical performance with sustainability goals.

Another significant trend is the advancement of miniaturised and high-efficiency chromatographic systems, such as ultra-high-performance liquid chromatography (UHPLC), micro-HPLC, and capillary electrophoresis. These techniques operate with reduced column dimensions and lower flow rates, resulting in decreased solvent consumption and faster analysis times. Jankech et al. (2024) emphasised that miniaturisation is a key strategy in achieving greener analytical methods, as it not only reduces environmental impact but also enhances analytical throughput and efficiency. The adoption of such technologies in pharmaceutical quality control laboratories is expected to increase, driven by both economic and environmental considerations.

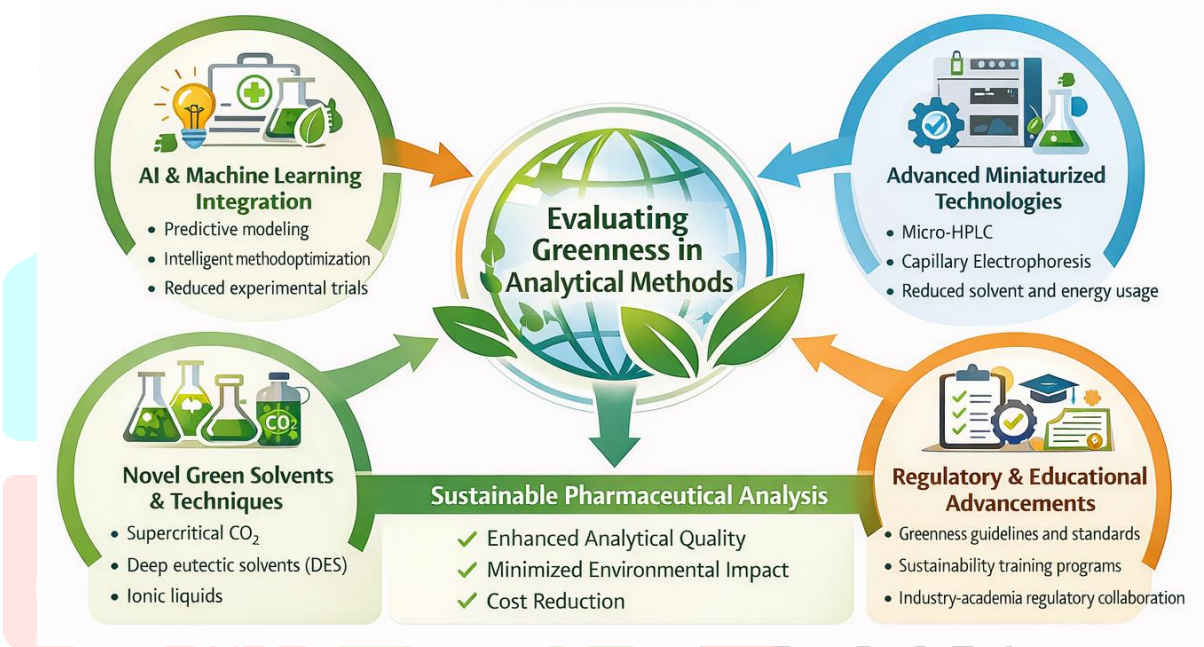

Figure 3. Future trends in green analytical chemistry for sustainable pharmaceutical analysis

The development and application of alternative separation techniques, such as supercritical fluid chromatography (SFC), also represent a promising direction for green analytical chemistry. SFC utilises supercritical carbon dioxide as the primary mobile phase, significantly reducing the need for organic solvents. Jambo et al. (2024) demonstrated the effectiveness of SFC-MS in the analysis of pharmaceutical impurities, highlighting its potential as a green alternative to conventional chromatographic methods. The low toxicity reduced solvent usage, and the high efficiency of SFC makes it an attractive option for future analytical applications, particularly in the analysis of complex pharmaceutical formulations. In parallel, the exploration of novel green solvents remains an active area of research. Deep eutectic solvents (DES) and ionic liquids offer unique physicochemical properties, including tunable polarity and low volatility, making them suitable for sustainable analytical applications. Although their widespread adoption is currently limited by cost and regulatory considerations, ongoing research is expected to address these challenges and expand their applicability in pharmaceutical analysis. The integration of green analytical chemistry with advanced sample preparation techniques is another important trend. The development of microextraction methods, automated sample preparation systems, and solvent-free analytical techniques is expected to significantly reduce the environmental impact of analytical workflows. Jankech et al. (2024) highlighted that reducing or eliminating sample preparation steps is a critical factor in enhancing method greenness, as it directly impacts solvent consumption and waste generation.

Digital and portable analytical technologies are also gaining prominence as tools for sustainable pharmaceutical analysis. Smartphone-based analytical systems, as demonstrated by de Souza Gomes et al. (2026), offer low-cost, rapid, and environmentally friendly alternatives for certain analytical applications. These technologies enable decentralised analysis, reducing the need for complex laboratory infrastructure and minimising resource consumption. Such approaches are particularly valuable in resource-limited settings and for on-site quality control.

Spectroscopic techniques are expected to play an increasingly important role in green analytical chemistry. Methods such as Fourier transform infrared (FTIR) spectroscopy provide solvent-free or low-solvent alternatives for pharmaceutical analysis. Cherniienko et al. (2024) highlighted that FTIR enables real-time monitoring of chemical processes with minimal environmental impact, making it a valuable tool for sustainable quality control. The integration of spectroscopic techniques with chemometric analysis further enhances their applicability by enabling the analysis of complex datasets with high accuracy.

The future of green analytical chemistry also involves the development of comprehensive, standardised frameworks for evaluating greenness. While existing tools such as NEMI, GAPI, AGREE, and BAGI provide valuable insights into method sustainability, there is a need for harmonised guidelines that integrate these metrics into regulatory frameworks. Bredendiek et al. (2026) emphasised that sustainability assessment should encompass not only environmental factors but also economic and practical considerations, ensuring that analytical methods are both green and feasible for routine use. Regulatory agencies are expected to play a crucial role in promoting the adoption of green analytical methods. The incorporation of sustainability criteria into regulatory guidelines would encourage pharmaceutical companies to develop and implement environmentally friendly analytical techniques. This shift would require collaboration between regulatory bodies, industry, and academia to establish standardised protocols for evaluating and validating green analytical methods.

Another important future direction is integrating green chemistry principles into pharmaceutical education and training. Increasing awareness and knowledge of GAC among analytical scientists is essential for driving the adoption of sustainable practices. Archana and Mani (2025) emphasised that education and training are key factors in promoting green analytical chemistry, as they enable researchers to design and implement environmentally responsible analytical methods. In the context of antidiabetic fixed-dose combinations, future research is expected to focus on developing universally applicable green analytical methods capable of simultaneously analysing multiple drugs with diverse physicochemical properties. The integration of AQbD, advanced optimisation techniques, and green solvents will be critical in achieving this goal. Additionally, the development of hybrid analytical systems that combine chromatographic and spectroscopic techniques may offer new opportunities for enhancing analytical performance while reducing environmental impact.

In conclusion, the future of green analytical chemistry in pharmaceutical quality control is characterised by the convergence of sustainability, technological innovation, and regulatory evolution. The integration of AI-driven optimisation, miniaturised analytical systems, alternative separation techniques, and green solvents is expected to significantly enhance the efficiency and sustainability of analytical methods. While challenges remain, continued research and collaboration across disciplines will play a vital role in advancing green analytical chemistry and facilitating its widespread adoption in pharmaceutical analysis.

10. CONCLUSION

The increasing emphasis on sustainability within the pharmaceutical industry has necessitated a paradigm shift in analytical practices, particularly in the domain of pharmaceutical quality control. RP-HPLC, despite being the gold standard analytical technique, has traditionally relied on solvent-intensive and energy-demanding methodologies that pose significant environmental and occupational concerns. The integration of Green Analytical Chemistry (GAC) principles into RP-HPLC method development offers a viable and scientifically robust pathway to address these challenges while maintaining analytical performance. The present review has comprehensively demonstrated that the adoption of green solvents, optimization of chromatographic conditions, and implementation of advanced frameworks such as

Analytical Quality by Design (AQbD) can significantly enhance the sustainability of RP-HPLC methods. Studies such as those by Al-Tannak and Hemdan (2023), Kalpana et al. (2025), and Ibrahim et al. (2024) have clearly shown that systematic optimization strategies can reduce solvent consumption, shorten analysis time, and improve method robustness without compromising accuracy or precision. Similarly, the application of greenness assessment tools, including NEMI, AGREE, GAPI, and BAGI, as highlighted by Jankech et al. (2024) and Mahgoub et al. (2025), provides a quantitative framework for evaluating and validating the environmental impact of analytical methods.

In the context of antidiabetic fixed-dose combinations, developing green RP-HPLC methods poses unique challenges due to the diverse physicochemical properties of the constituent drugs. However, the studies reviewed indicate that these challenges can be effectively addressed through the integration of green chemistry principles with statistical optimisation techniques. The findings of Haneef and Khan (2023) and Barzani et al. (2025) further emphasise the need for continued efforts to improve the greenness of analytical methods for antidiabetic drugs, as many existing methods still rely on conventional solvents and practices. The review also highlights the growing importance of alternative analytical techniques, such as spectrophotometric, spectrofluorimetric, and spectroscopic methods, in supporting sustainable pharmaceutical analysis. Techniques such as FTIR spectroscopy and smartphone-based analytical systems, as reported by Cherniienko et al. (2024) and de Souza Gomes et al. (2026), demonstrate the potential to reduce solvent use and simplify analytical workflows. While these methods may not fully replace chromatographic techniques, they offer valuable complementary approaches that contribute to overall sustainability.

Despite these advancements, several challenges remain in the widespread adoption of green analytical methods, including limitations in solvent selection, analytical complexity, sensitivity requirements, and regulatory acceptance. Addressing these challenges requires a multidisciplinary approach that combines advances in analytical chemistry, process optimisation, regulatory frameworks, and education. The integration of emerging technologies such as artificial intelligence, machine learning, and miniaturised analytical systems is expected to further enhance the development of sustainable analytical methods, as highlighted by Barzani et al. (2026). From a broader perspective, the transition toward green analytical chemistry is not merely an environmental necessity but also an opportunity to improve the efficiency, cost-effectiveness, and safety of pharmaceutical analysis. The adoption of sustainable analytical practices can lead to reduced operational costs, improved laboratory safety, and enhanced compliance with environmental regulations. However, achieving this transition requires a concerted effort from academia, industry, and regulatory bodies to promote awareness, develop standardised guidelines, and facilitate the implementation of green methodologies. In conclusion, green RP-HPLC represents a promising and necessary evolution in pharmaceutical quality control, particularly for the analysis of complex antidiabetic fixed-dose combinations. The integration of green chemistry principles, advanced optimisation strategies, and comprehensive greenness evaluation metrics provides a strong foundation for developing analytical methods that are both environmentally sustainable and analytically robust. Continued research, technological innovation, and regulatory support will be essential to fully realise the potential of green analytical chemistry and to ensure its successful implementation in routine pharmaceutical practice.

REFERENCES

1. Al-Mutairi, A. A., et al. (2026). Development and validation of green spectrophotometric methods for simultaneous determination of etoricoxib and tramadol. *Scientific Reports, 16*(1).
2. Alqahtani, A., et al. (2025). Development and validation of a green spectrofluorimetric method for nateglinide quantification: Utilizing AR87 as a probe with Box–Behnken optimization and quantum mechanical calculations. *Luminescence, 40*(1), e70076.
3. Al-Tannak, N. F., & Hemdan, A. (2023). Eco-friendly HPLC method by using response surface design to measure a combination of three antidiabetic drugs. *Reviews in Analytical Chemistry, 42*(1), 20230063.
4. Archana, K., & Mani, S. (2025). Advancing green chemistry in antiviral therapeutics: A comprehensive review. *Current Drug Research Reviews, 17*(1), 10–18.
5. Barzani, H. A. H., et al. (2025). The analytical evolution of glibenclamide (glyburide) antidiabetic agents: Analytical tools, challenges, and future trends. *Critical Reviews in Analytical Chemistry*, 1–29.
6. Barzani, H. A. H., et al. (2026). Analytical approaches for the determination of lidocaine and its metabolites: A comprehensive insight. *Critical Reviews in Analytical Chemistry*, 1–37.
7. Barzani, H. A. H., et al. (2026). Comprehensive insight into analytical strategies for the determination of zolpidem and its metabolites in different matrices. *Critical Reviews in Analytical Chemistry*, 1–23.
8. Barzani, H. A. H., et al. (2026). Analytical techniques for the quantification of pravastatin and its metabolites in various matrices: A comprehensive review. *Biomedical Chromatography, 40*(2), e70335.
9. Bhatia, N. M., et al. (2026). Design of experiments based reversed-phase high-performance liquid chromatography method for estimation of metformin and glimepiride in combined dosage forms. *The Thai Journal of Pharmaceutical Sciences, 50*(1), 4.
10. Bredendiek, F., et al. (2026). Sustainability in analytical chemistry illustrated by pharmaceutical nitrosamine testing. *Electrophoresis, 47*(2), 175–184.
11. Cherniienko, A., et al. (2024). IR-EcoSpectra: Exploring sustainable ex situ and in situ FTIR applications for green chemical and pharmaceutical analysis. *Journal of Pharmaceutical Analysis, 14*(9), 100951.
12. de Souza Gomes, B. R., et al. (2026). Smartphone-based digital imaging for rapid and green quantification of aspartame in lemon-flavored soft drinks. *Food Chemistry, 499*, 147312.
13. Haneef, J., & Khan, M. D. (2023). Liquid chromatographic methods for the analysis of canagliflozin: Concise overview and greener assessment. *Analytical Methods, 15*(36), 4627–4639.
14. Ibrahim, A. E., et al. (2024). Leveraging green chromatography in quality-by-design-driven study for the simultaneous analysis of essential single-pill antidiabetic drug combinations. *Journal of Separation Science, 47*(24), e70063.
15. Jambo, H., et al. (2024). Comprehensive analysis of vitamin D_3 impurities in oily drug products using supercritical fluid chromatography–mass spectrometry. *Drug Testing and Analysis, 16*(7), 692–707.
16. Jankech, T., et al. (2024). Current green capillary electrophoresis and liquid chromatography methods for analysis of pharmaceutical and biomedical samples (2019–2023)—A review. *Analytica Chimica Acta, 1323*, 342889.
17. Kalpana, P., et al. (2025). Green metrics-based HPLC method for quantification of three antidiabetic drugs in tablet dosage form using Box–Behnken design. *Accreditation and Quality Assurance, 30*(2), 141–152.
18. Kabil, N. A. S., et al. (2025). Green HPLC analysis of anti-diabetic and gastric suppressant drugs: A sustainable approach. *Microchemical Journal, 215*, 114244.

19. Konari, S. N., & Jacob, J. T. (2015). Stability indicating validated RP-HPLC technique for the analysis of multicomponent anti-diabetic drug combinations in pharmaceutical dosage forms. *Karbala International Journal of Modern Science, 1*(1), 39–48.
20. Lustosa, I. A., & Kogawa, A. C. (2024). An overview of the analytical methods for products based on clindamycin and eco-efficiency perspectives. *Current Pharmaceutical Design, 30*(40), 3155–3163.
21. Magdy, G., et al. (2023). Analytical quality-by-design approach for development and validation of HPLC method for the simultaneous estimation of omarigliptin, metformin, and ezetimibe: Application to human plasma and dosage forms. *BMC Chemistry, 17*(1), 45.
22. Mahgoub, S. M., et al. (2025). Green, innovative oral capsules of three anti-Parkinson's drugs: Formulation, in vitro evaluation, and robust RP-HPLC validation. *Analytical Methods, 17*(30), 6215–6230.
23. MM, Z. S., et al. (2021). Validated green chromatographic methods for determination of amlodipine and celecoxib in presence of methylacetophenone. *Bioanalysis, 13*(12), 969–983.