

Eco-Friendly AQbD-Driven HPLC: A Sustainable Approach to Method Development and Validation Using Green Chemistry

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ABSTRACT

The integration of Analytical Quality by Design (AQbD) with Green Analytical Chemistry (GAC) represents a transformative approach in High-Performance Liquid Chromatography (HPLC) method development and validation. This review aims to provide a comprehensive overview of how AQbD frameworks can be systematically combined with GAC principles to achieve robust, reproducible, and environmentally sustainable analytical methods. AQbD facilitates method optimization through tools such as risk assessment, Design of Experiments (DoE), and Method Operable Design Region (MODR) establishment, while GAC focuses on minimizing hazardous solvent use, energy consumption, and waste production. This review evaluates peer-reviewed studies published between 2022 and mid-2025 that demonstrate AQbD-based HPLC and UPLC methods employing eco-friendly solvents, particularly ethanol and water, and assessing environmental sustainability using green metrics including AGREE, GAPI, AMGS, and Analytical Eco-Scale. Case studies on pharmaceutical analytes such as metronidazole, nicotinamide, irbesartan, metformin, and empagliflozin illustrate the practical implementation of these approaches, with several studies reporting high green scores and regulatory alignment with ICH guidelines. Despite significant progress, challenges persist, including inconsistent application of greenness metrics, limited availability of green solvent alternatives, and the need for integrated software tools that combine AQbD and GAC evaluation. This review highlights key methodological gaps and future opportunities, including extending AQbD-GAC approaches to complex matrices and integrating Artificial Intelligence (AI) to enhance optimization and sustainability. Overall, the convergence of AQbD and GAC not only ensures analytical reliability and regulatory compliance but also aligns with global sustainability goals and emerging eco-conscious scientific practices.

Keywords: Design of Experiments (DoE), Regulatory Compliance (RC), Green analytical chemistry (GAC), Critical Quality Attributes (CQAs), Analytical Target Profile (ATP).

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INTRODUCTION

The purpose of this review is to explore the synergistic integration of Analytical Quality by Design (AQbD) with Green Analytical Chemistry (GAC) principles in the context of High-Performance Liquid Chromatography (HPLC) method development and validation. The main aim of the review to demonstrate how AQbD offers systematic, robust method design while green chemistry ensures minimal environmental impact yielding high-performance yet sustainable analytical protocols. In terms of scope, this article covers recent developments from approximately

2022 to mid 2025. It focuses on applications of AQbD framework HPLC/UPLC methods that leverage ecofriendly solvents (such as ethanol) and evaluate environmental performance using established greenness metrics (e.g., AGREE, GAPI, AMGS, NQS). The review emphasises pharmaceutical analysis, including stability indicating and nanoparticle assays, to illustrate practical method implementation and validation under International Council for Harmonisation (ICH) guidelines (Kiwfo *et al.*, 2025).2025)

The background of the concept of Green Analytical Chemistry evolved from the broader green chemistry movement, guided by 12 core principles targeting waste prevention, safer solvent use, energy efficiency, and realtime monitoring (Gałuszka *et al.*, 2015). These principles have been adapted to analytical practices, motivating researchers to minimize toxic solvent usage especially



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prevalent in traditional HPLC methods using acetonitrile or methanol and to incorporate greener alternatives such as ethanol, water, or biodegradable solvents (El Deeb, 2024).

Meanwhile, Analytical Quality by Design (AQbD) provides a structured framework for method development: defining the Analytical Target Profile (ATP), identifying Critical Method Parameters (CMPs), and using design of experiments (e.g. central composite designs) to optimize robustness and reproducibility. Combining AQbD with GAC yields methods that are simultaneously robust, scientifically sound, and environmentally responsible.

Recent literature presents several high-impact case studies demonstrating the integration of Analytical Quality by Design (AQbD) with Green Analytical Chemistry (GAC) in High-Performance Liquid Chromatography (HPLC) method development. The study described an AQbD-driven RP-HPLC method for quantifying irbesartan in chitosan nanoparticles, employing an ethanol-sodium acetate mobile phase, central composite design optimization, and environmental risk assessment to yield an eco-friendly validated method aligned with green principles (Basha *et al.*, 2024a). In a study, an environmentally friendly RP-HPLC PDA method was established for the simultaneous estimation of metronidazole and nicotinamide, optimized using AQbD principles. The greenness assessment using AGREE (0.75) and NQS (~63%) indices confirmed the method's high sustainability while maintaining analytical quality and alignment with the United Nations Sustainable Development Goals (UN-SDGs) (Kiwfo *et al.*, 2025). Additionally, comprehensive guidance has been provided on transitioning conventional HPLC/UHPLC methods toward greener practices, emphasizing solvent reduction, the adoption of core-shell or sub-2 μm columns, faster analysis times, and the use of energy-efficient instruments to minimize environmental impact (Armenta *et al.*, 2008). Furthermore, recent reviews highlight the dual advantage of merging GAC and QbD frameworks in analytical method development, emphasizing the utility of tools such as HPLC-EAT, AMVI, and GAPI in combination with Design of Experiments (DOE) for designing greener pharmaceutical analysis methods (El Deeb, 2024; Tobiszewski *et al.*, 2015; Verma *et al.*, 2021). Despite the growing interest in integrating GAC with AQbD in method development, there remains a lack of comprehensive reviews that critically evaluate and compare eco-friendly AQbD-based HPLC approaches, especially concerning the application of standardized greenness metrics like AGREE, GAPI, AMGS, and NEMI, in alignment with the UN-SDGs (Mallet *et al.*, 2020; Sheldon, 2000). Therefore, this review aims to critically analyze recent AQbD-driven eco-friendly HPLC methods, evaluate the application of greenness assessment tools, highlight the combined benefits of GAC and QbD frameworks, offer practical guidance for adopting sustainable analytical methodologies, and identify

research gaps to guide future advancements in green analytical method development.

METHODOLOGY

This review was conducted using a systematic literature search of peer-reviewed articles published between January 2022 and June 2025. Databases searched included Scopus, PubMed, Web of Science, and Google Scholar. Keywords used were "Analytical Quality by Design," "Green Analytical Chemistry," "HPLC method development," "eco-friendly solvents," "AGREE," "GAPI," and "sustainable chromatography." Inclusion criteria comprised original research, reviews, and regulatory publications focusing on AQbD and GAC integration in HPLC or UPLC method development. Articles without experimental validation or environmental assessment were excluded (Tobiszewski *et al.*, 2015). The included studies were evaluated based on methodological rigor, relevance to pharmaceutical analysis, and the use of recognized green metrics (e.g., AGREE, GAPI, NEMI, Eco-Scale, etc).

This review presents methodological insights derived from an extensive analysis of the principles and practices involved in Analytical Quality by Design (AQbD) and its integration with Green Analytical Chemistry (GAC) in High-Performance Liquid Chromatography (HPLC) (Trivedi *et al.*, 2023). These insights aim to guide researchers and analysts in implementing systematic, science-based, and environmentally responsible approaches to method development and validation (Mallet *et al.*, 2020). The AQbD Strategic principles and methodological insights are follows (Verma *et al.*, 2021).

Defining the Analytical Target Profile (ATP)

The ATP outlines the intended purpose of the analytical method and sets predefined performance criteria. This includes accuracy, precision, linearity, robustness, sensitivity, and eco-friendliness. Establishing the ATP ensures that the method meets both regulatory expectations and green objectives (Beg *et al.*, 2019).

Identification of Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs)

Methodological frameworks emphasize identifying CQAs (e.g., resolution, retention time, peak symmetry) and associating them with CMPs such as flow rate, mobile phase composition, column temperature, and detection wavelength. These parameters are assessed for their influence on method performance and greenness (Nandy and Roy, 2020) (Trivedi *et al.*, 2023).

Risk Assessment

AQbD utilizes risk assessment tools such as Ishikawa diagrams showing in Figure 1: Ishikawa (Fishbone Diagram for analytical errors), Failure Mode and Effects Analysis (FMEA), and risk matrices to prioritize variables that significantly affect method

quality (Mukherjee and Mandal, 2023). This ensures that resource-efficient optimization and minimizes experimental burden while enhancing method robustness (Lionta *et al.*, 2022).

Design of Experiments (DoE)

DoE is a central AQbD tool that enables the systematic evaluation of multiple factors and their interactions. Techniques such as factorial design, Box-Behnken, or central composite design help in identifying the optimal design space for CMPs while also reducing the number of trials. This aligns with both cost efficiency and environmental goals (El Deeb, 2024).

Establishing the Method Operable Design Region (MODR)

MODR represents the multidimensional region in which the method delivers acceptable performance. It allows flexibility for minor adjustments without revalidation (Rios and Hernandez, 2017). Establishing MODR enhances method lifecycle management and aligns with ICH Q14 guidelines (ICH, 2023). And explaining different Key guidelines in pharmaceutical development (Figure 2).

Validation under AQbD Framework

Validation parameters such as accuracy, precision, linearity, specificity, robustness, LOD, and LOQ are assessed within the optimized MODR. AQbD-based validation ensures methods are resilient to variability while complying with regulatory guidelines like ICH Q2(R2) (Trivedi *et al.*, 2023). And shows Analytical method development process in Figure 3 (ICH, 2021).

Incorporation of Green Analytical Chemistry Tools

A key methodological advancement in recent years is the integration of environmental assessment metrics. Tools such as:

- **AGREE (Analytical Greenness calculator):** Provides a holistic, 12-principle-based sustainability score (Samanidou and Papadoyannis, 2021),
- **GAPI (Green Analytical Procedure Index):** Visual tool for quick green assessment across method stages (Gałaszka *et al.*, 2013),
- **Analytical Eco-Scale:** Penalizes non-green practices, generating a semi-quantitative sustainability score.

These tools help method developers quantify and compare the environmental impact of different analytical workflows (Kiwfo *et al.*, 2025; Gałaszka *et al.*, 2012) and in addition to AGREE, GAPI, and Analytical Eco-Scale, several Green Analytical Chemistry (GAC) tools have been developed to assess and improve the environmental sustainability of analytical methods. Below is a curated comparative list of commonly used and emerging various tool are depicted in Table 1 (Chakraborty and Jayaseelan, 2023).

Software and Automation

Use of advanced software such as Design-Expert, Minitab, or Fusion QbD® allows rapid modelling, optimization, and validation of methods while minimizing reagent and energy consumption-enhancing sustainability and reproducibility (O'Brien and Patel, 2023).

Lifecycle and Continual Improvement

An AQbD-driven method is not static. The lifecycle approach encourages ongoing monitoring and updates as new technologies or greener solvents become available (Singh and Singh, 2020). This promotes regulatory flexibility and continuous improvement in sustainability (El Deeb, 2024).

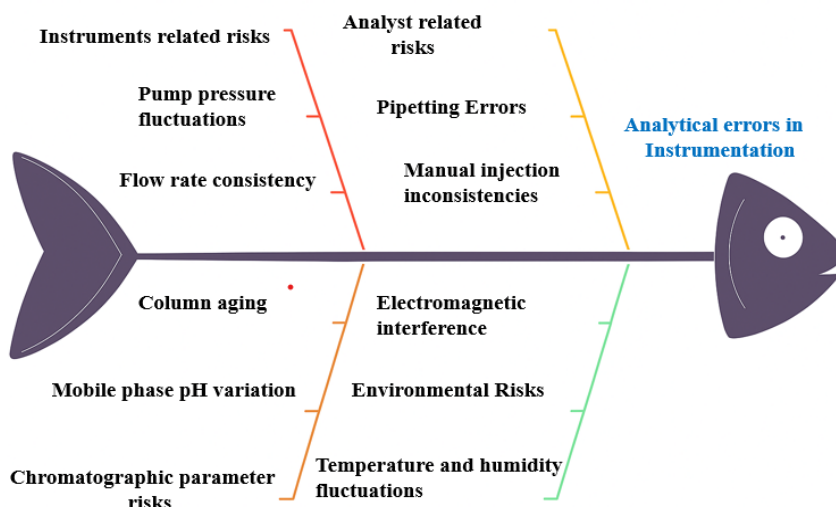


Figure 1: Ishikawa (Fishbone) Diagram for analytical errors.

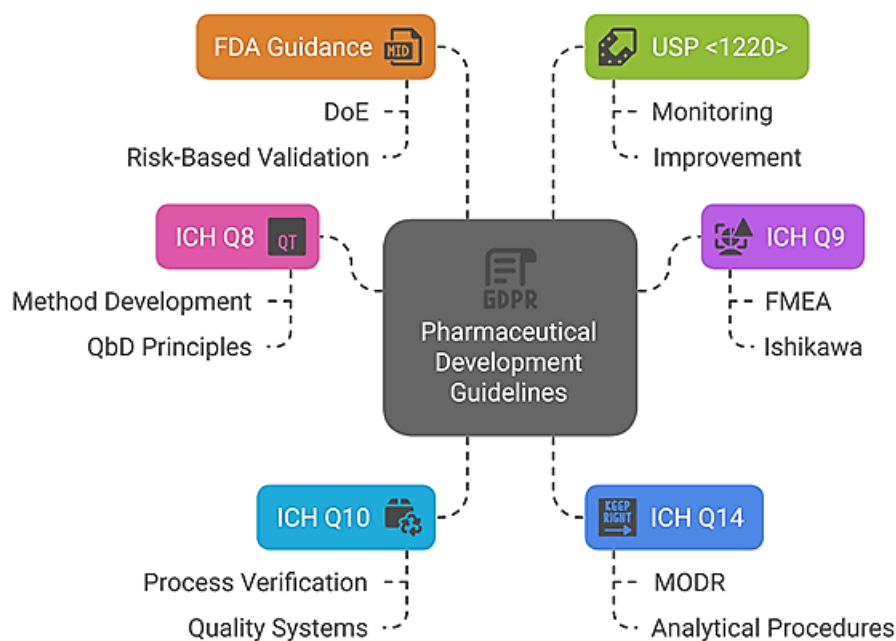


Figure 2: Key guidelines in pharmaceutical development.

BODY OF THE METHOD DEVELOPMENT

Evolution of Analytical Quality by Design (AQbD) in HPLC

Analytical Quality by Design (AQbD) emerged as a paradigm shift from empirical method development toward a systematic, science-based framework ensuring robustness, reliability, and regulatory compliance. Initially applied in pharmaceutical process development, AQbD has evolved into a key strategy in analytical method design, particularly for High-Performance Liquid Chromatography (HPLC) (Beg *et al.*, 2019). The integration of AQbD in HPLC ensures a comprehensive understanding of Critical Quality Attributes (CQAs), Critical Method Parameters (CMPs), and Method Operable Design Region (MODR), thereby improving method robustness and lifecycle flexibility (Trivedi *et al.*, 2023) and comparatively AQbD is more accurate and Robust shows Table 2: QbD vs Traditional Method Development in HPLC.

Framework and Tools of AQbD in HPLC

The AQbD framework encompasses the following elements:

- Analytical Target Profile (ATP),
- Risk Assessment,
- Design of Experiments (DoE),
- MODR determination,
- Control Strategy and Lifecycle Management (Reichenbach and Carr, 2022).

Modern software tools like Design Expert, Fusion QbD, and Minitab allow researchers to model the interactions of parameters efficiently, enabling rapid method optimization with minimal trial-and-error (El Deeb, 2024). These tools help visualize parameter spaces, quantify interactions, and define MODRs and explains Figure 4: Optimizing method development through structured framework and analysis (Armenta *et al.*, 2008).

Despite its benefits, challenges remain regarding the uniform implementation of AQbD across laboratories due to the required statistical and regulatory expertise. Moreover, few open-access tools exist for AQbD integration with green chemistry, indicating a key methodological gap (Płotka-Wasyłka, 2018).

Integration of Green Analytical Chemistry Principles

The pressing need to reduce the environmental footprint of analytical methods has driven the merger of green chemistry principles with AQbD. The 12 Principles of Green Analytical Chemistry (Galuszka *et al.*, 2013) encourage the reduction of hazardous solvents, energy, and waste while promoting miniaturization and automation (Bakshi and Singh, 2022).

Key trends include:

- Replacing conventional solvents (e.g., acetonitrile) with bio-based or less toxic alternatives (e.g., ethanol, water) (Smith *et al.*, 2014),
- Miniaturization of techniques (e.g., micro-HPLC),
- Solvent-reducing methods like UPLC, SFC, and MEKC.

The AQbD-GAC hybrid approach enables not only robustness and regulatory alignment but also sustainability.

Table 1: Comparative Overview of GAC Assessment Tools.

Tool	Full Name	Type	Key Features	Strengths	Limitations
NEMI	National Environmental Methods Index	Qualitative	Uses a pictogram to assess waste, pH, hazard, and persistence.	Simple, easy to interpret	Binary output; lacks granularity and quantification.
GAPI	Green Analytical Procedure Index	Semi-quantitative	Colored pentagram covering 15 method stages.	Visual, comprehensive across method lifecycle	Subjective; does not provide a numerical score.
Eco-Scale	Analytical Eco-Scale	Quantitative	Penalty point-based scoring out of 100.	Numeric, easy comparison among methods	Limited to reagent-based assessment.
AGREE	Analytical Greenness Metric	Quantitative	Circular pictogram; evaluates all 12 GAC principles with overall score (0-1).	Integrates all 12 GAC principles; visual + numeric outputs	Requires full data input; newer, still gaining adoption.
AGREEprep	Sample Preparation Greenness Metric	Quantitative	Focuses on greenness of sample preparation only.	Sample-prep specific; aligns with AGREE framework	Does not assess entire method.
HPLC-EAT	HPLC Environmental Assessment Tool	Quantitative	Specifically for HPLC; calculates solvent-related impact.	Tailored to chromatographic methods	Limited to HPLC; not universal for all techniques.

Table 2: QbD vs Traditional Method Development in HPLC.

Feature	Traditional Approach	QbD Approach
Optimization Strategy	Trial-and-error	DoE-based modelling
Risk Management	Limited	Comprehensive risk assessment
Regulatory Flexibility	Low	High (MODR application)
Method Transferability	Prone to failure	Highly robust
Revalidation Requirement	Frequent	Minimized

Green Metrics and Evaluation Tools

Several tools have been developed to assess and visualize the greenness of analytical methods: These tools support the objective evaluation of environmental performance and are increasingly used in AQbD-based method publications (Pena-Pereira *et al.*, 2020; Kiwfo *et al.*, 2025).

Critical Analysis of Literature Trends

Recent literature reflects a clear shift toward sustainable method developments in AQbD and GAC integration in 2015-2019: AQbD focused primarily on robustness and precision, 2020-2023: Increasing inclusion of green chemistry metrics and visual tools, 2024 onward: Convergence of regulatory (ICH Q14) and sustainability frameworks (Basha *et al.*, 2024b). Analytical quality-by-design-based RP-HPLC method development and validation for irbesartan in chitosan nanoparticles.

However, despite the growing awareness, the use of green metrics in method validation is still inconsistent. Many studies claim to be green or eco-friendly but do not quantify or justify these claims using AGREE or GAPI. Additionally, the application of AQbD in bioanalytical methods and complex matrices (e.g., biological fluids, environmental samples) remains limited, creating a research gap for real-world, matrix-rich sustainable analysis (Verma *et al.*, 2021).

Gaps and Future Directions

Key gaps identified through this review include

Lack of standardization in applying green metrics in regulatory submissions, Limited accessibility to integrated AQbD-GAC software, Scarce training and awareness in academic/research

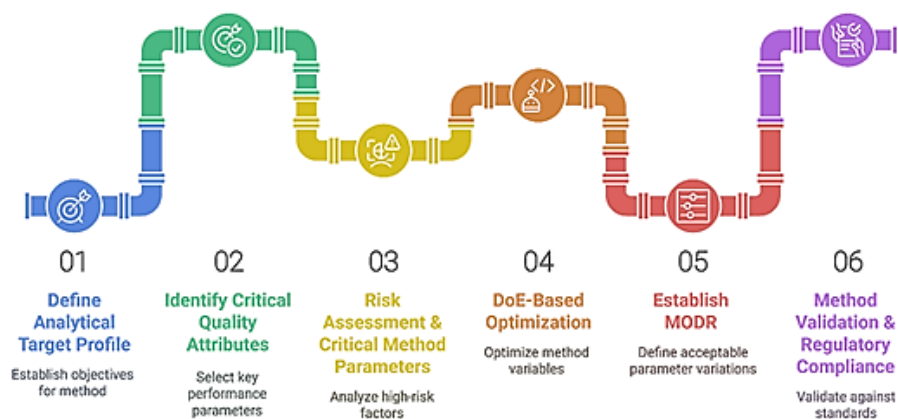


Figure 3: Analytical method development process.

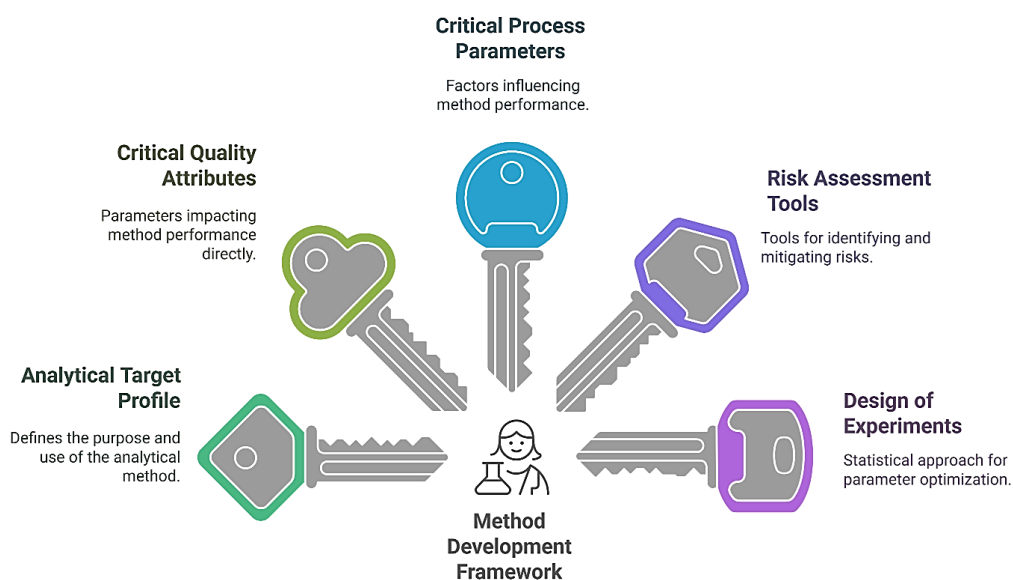


Figure 4: Optimizing method development through structured framework and analysis.

labs regarding MODR or green evaluation tools, Underexplored potential of AQbD for real-time environmental monitoring, clinical diagnostics, and point-of-care testing.

Future research should focus on: (Pena-Pereira *et al.*, 2020)

Developing open-access AQbD-GAC integration platforms

- Applying green AQbD methods in multi-analyte, stability-indicating, and degradant profiling studies.
- Promoting interdisciplinary collaboration between analytical chemists, environmental scientists, and regulatory experts.

DISCUSSION

This review emphasizes the significant strides made in applying Analytical Quality by Design (AQbD) to High-Performance Liquid Chromatography (HPLC), particularly when integrated

with Green Analytical Chemistry (GAC) principles to create robust, efficient, and environmentally responsible analytical methods. AQbD facilitates systematic method development through the identification of critical quality attributes, risk assessment, and design of experiments, while the incorporation of GAC ensures alignment with sustainability goals (Beg *et al.*, 2019; El Deeb, 2024). Tools such as AGREE, GAPI, and the Analytical Eco-Scale have been increasingly used to quantify and visualize the environmental impact of analytical procedures, offering insight into solvent toxicity, energy use, and waste generation (Pena-Pereira *et al.*, 2020; Galuszka *et al.*, 2012). Despite these advancements, challenges persist in terms of inconsistent application and standardization of green metrics, limited availability of universally acceptable green solvents, and the high cost or limited accessibility of AQbD-enabling software in academic and resource-constrained laboratories (Trivedi *et al.*, 2023; Kiwfo *et al.*, 2025). Additionally, the application of AQbD-GAC methods in complex biological or environmental

matrices remains limited, highlighting a gap in method translation to real-world scenarios. Moving forward, research should focus on developing integrated AQbD-GAC assessment frameworks, automating green evaluation tools within analytical software, and expanding the use of greener sample preparation strategies such as solid-phase microextraction and deep eutectic solvents. There is also a need to promote broader application in diverse matrices and enhance educational outreach to embed sustainability principles into early-stage analytical training (ICH, 2023). Collectively, these efforts will help bridge current gaps and advance the implementation of eco-friendly, quality-centric chromatographic science (Horváth, 2020).

CONCLUSION

The integration of Analytical Quality by Design (AQbD) with Green Analytical Chemistry (GAC) in HPLC promotes the development of robust, regulatory-compliant, and environmentally sustainable methods. AQbD ensures systematic method optimization using tools like DoE and risk assessment, while GAC principles, assessed through metrics such as AGREE, GAPI, and Eco-Scale, help minimize solvent use, energy consumption, and hazardous waste. Although recent studies highlight progress in balancing analytical performance with environmental responsibility, challenges like inconsistent application of green metrics, limited solvent options, and underrepresentation of complex matrices remain. This combined approach supports global sustainability goals and regulatory demands, with emerging AI and machine learning tools offering further opportunities for method optimization and efficiency.

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ABBREVIATIONS

AI: Artificial Intelligence; **ATP:** Analytical Target Profile; **CPV:** Continuous Process Verification; **CQAs:** Critical Quality Attributes; **CPPs:** Critical Process Parameters; **DoE:** Design of Experiments; **EMA:** European Medicines Agency; **FDA:** Food and Drug Administration; **FMEA:** Failure Mode and Effects Analysis; **GF:** Fluorescent indicators and gypsum used as a binder; **GAC:** Green Analytical Chemistry; **HPLC:** High-Performance Liquid Chromatography; **NEMI:** National Environmental Methods Index; **NQS:** National Quality Score; **ICH:** International Council for Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of quantification; **SFC:** Supercritical Fluid Chromatography; **MEKC:** Micellar Electrokinetic Chromatography; **SDGs:** Sustainable Development Goals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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