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Review Article

Green HPLC Method Development: Recent Advances, Challenges, and Future Perspectives

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High performance liquid chromatography (HPLC) is the most widely used analytical separation technique in pharmaceutical, environmental, food, and clinical laboratories, but conventional practice depends heavily on large volumes of toxic, petroleum-derived solvents such as acetonitrile and methanol. Regulatory pressure, solvent shortages, disposal costs, and laboratory safety concerns have driven the emergence of Green HPLC, an extension of the twelve principles of green chemistry to liquid chromatographic method development. This review consolidates recent advances, including substitution of hazardous mobile phase solvents with greener alternatives such as ethanol, carbonate esters, and bio-derived ethers; adoption of ultra-high-performance liquid chromatography (UHPLC) with sub-2 micron and superficially porous particles to reduce solvent and energy consumption; column miniaturization; and integration of Analytical Quality by Design (AQbD) with Design of Experiments (DoE) to rationally optimize critical method parameters while minimizing environmental burden. Greenness assessment tools, namely the National Environmental Methods Index (NEMI), Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and the Analytical GREEnness (AGREE) calculator, are reviewed as quantitative frameworks for benchmarking method sustainability alongside validation parameters defined in ICH Q2(R2). Persistent challenges are discussed, including higher viscosity and UV cut-off of green solvents, regulatory inertia toward non-compendial methods, instrument cost, and the difficulty of simultaneously optimizing performance, cost, and greenness. Finally, the review outlines future perspectives, including artificial intelligence-assisted optimization and convergence toward "white analytical chemistry," balancing environmental, economic, and analytical considerations. These developments indicate that green HPLC is transitioning from a niche interest into a mainstream expectation for analytical quality control.

Keywords: Green analytical chemistry, Green HPLC, AGREE, GAPI, Quality by Design, sustainable chromatography, green solvents.

INTRODUCTION

High performance liquid chromatography (HPLC) remains the backbone of analytical quality control in pharmaceutical, food, environmental, and clinical laboratories owing to its versatility, sensitivity, and broad applicability to polar and non-polar analytes alike. A typical pharmacopoeial HPLC assay, however, consumes substantial volumes of organic solvents over its operational lifetime, most commonly acetonitrile and methanol, both of which are derived from non-renewable petrochemical feedstocks, are flammable, and pose recognized toxicological and environmental hazards. The cumulative effect of thousands of laboratories running routine reversed-phase separations around the clock has placed liquid chromatography among the more solvent- and energy-intensive techniques in the analytical sciences. Green chemistry, formalized by Anastas and Warner through twelve guiding principles, originally addressed synthetic and process chemistry; its translation into the analytical laboratory gave rise to Green Analytical Chemistry (GAC), a discipline concerned with reducing the environmental footprint

of measurement science itself, spanning sample collection, preparation, separation, detection, and waste disposal. Within GAC, Green HPLC refers specifically to the redesign of liquid chromatographic methods so that they minimize hazardous reagent consumption, energy use, and waste generation without compromising the validity, sensitivity, and robustness required of a pharmacopeial or regulatory method. The urgency behind this shift stems from several converging pressures. Regulatory and standard-setting bodies increasingly expect sustainability considerations alongside traditional validation criteria. Solvent costs and intermittent acetonitrile shortages, most notably the global supply disruption observed in the early 2010s, exposed the fragility of chromatography laboratories that depend on a narrow set of petrochemical solvents. Occupational health concerns associated with chronic exposure to acetonitrile and chlorinated solvents have further reinforced calls for safer alternatives. Finally, the broader sustainability agenda embedded in institutional and corporate environmental, social, and governance (ESG) commitments has begun to influence how analytical laboratories evaluate and report their methods. This review aims to consolidate the current state of green HPLC method development. It examines the principles underpinning green analytical chemistry as applied to liquid chromatography, surveys recent advances in green mobile phase solvents, column and instrument miniaturization, and rational method optimization strategies such as Analytical Quality by Design (AQbD), reviews the quantitative greenness assessment tools used to benchmark sustainability, discusses the practical challenges that continue to limit widespread adoption, and outlines emerging directions including artificial intelligence-assisted optimization and the convergence of green, blue, and white analytical chemistry frameworks.

Principles of Green Analytical Chemistry Relevant to HPLC

The twelve principles of green analytical chemistry, as adapted by Galuszka, Migaszewski and Namiesnik, translate the broader green chemistry framework into laboratory practice. Several principles bear directly on chromatographic method design: preference for direct analytical techniques that avoid sample derivatization; minimization of sample and reagent quantities; use of safer solvents and reagents in place of hazardous ones; integration and automation of analytical steps to reduce reagent consumption; avoidance of unnecessary derivatization; minimization of waste generation with proper treatment; multi-analyte or multi-parameter methods in preference to single-analyte procedures; reduced energy consumption; reagents obtained from renewable sources; elimination or reduction of toxic reagents; increased operator safety; and generation of methods compatible with real-time monitoring. In the specific context of HPLC, the principles most frequently addressed by published green methods relate to solvent substitution, miniaturization and energy reduction, and waste minimization through shorter run times and reduced injection volumes.

MATERIALS AND METHODS

This article is a narrative literature review rather than an original experimental study; accordingly, this section describes the literature search strategy and the conceptual framework used to organize the reviewed material rather than laboratory materials and procedures.

Literature Search Strategy

A structured search of the peer-reviewed literature was conducted using PubMed, ScienceDirect, Springer Link, MDPI, and Google Scholar, supplemented by full-text retrieval from open-access repositories. Search terms included combinations of “green HPLC,” “green analytical chemistry,” “sustainable liquid chromatography,” “green solvent mobile phase,” “AGREE,” “GAPI,” “Analytical Eco-Scale,” “Quality by Design HPLC,” and “white analytical chemistry.” Priority was given to articles published between 2016 and 2026 to capture the most recent developments, while foundational papers establishing key concepts, metrics, and solvent systems were included regardless of publication date. Articles were screened for relevance to liquid chromatographic method development, sustainability assessment, and pharmaceutical or environmental application, and the resulting body of literature was synthesized thematically rather than systematically pooled.

Organizing Framework

The reviewed literature is organized under four broad themes that correspond to the major strategies by which liquid chromatographic methods are made greener: (i) substitution of hazardous mobile phase solvents with safer alternatives, (ii) instrumental and column-based miniaturization to reduce solvent and energy consumption, (iii) rational, model-based method optimization through AQbD and Design of Experiments to minimize trial-and-error solvent use, and (iv) quantitative greenness assessment using standardized metrics. Applications of these strategies in pharmaceutical, food, and environmental analysis are then discussed, followed by a critical appraisal of persistent challenges and a forward-looking discussion of emerging tools and frameworks.

RESULTS AND DISCUSSION

1. Green Mobile Phase Solvents in Reversed-Phase HPLC

Acetonitrile and methanol dominate reversed-phase HPLC because of their favorable UV transparency, low viscosity, and well-characterized chromatographic behavior. Both, however, are flammable, toxic upon chronic exposure, and derived from non-renewable feedstocks; acetonitrile additionally generates hydrogen cyanide upon combustion or improper incineration. A substantial body of recent work has focused on identifying solvents that retain acceptable chromatographic performance while reducing toxicity, flammability hazards, and environmental persistence.

Ethanol has emerged as the most extensively validated green alternative to acetonitrile and methanol in reversed-phase separations. A recent comprehensive analysis of 135 published applications of ethanol-water mobile phases in RP-HPLC between 1990 and the present confirms its established and growing role as a green modifier, despite its comparatively higher viscosity and stronger UV absorbance at low wavelengths.1 Ethanol is renewable when derived from fermentation, has low toxicity, and is biodegradable, making it attractive for both pharmaceutical and food-related applications. Other carbon-neutral or bio-derived solvents under active investigation include acetone, ethyl lactate, propylene carbonate, dimethyl carbonate, and Cyrene (dihydrolevoglucosenone), each offering a different balance of polarity, viscosity, UV cut-off, and miscibility with water.1 For normal-phase separations, particularly of non-polar and non-volatile analytes such as lipids, alternative solvents including hexamethyldisiloxane, cyclopentyl methyl ether (CPME), 2-methyltetrahydrofuran (2-MeTHF), and d-limonene have been shown to successfully replace n-heptane and chloroform without loss of selectivity, while substantially reducing the toxicological burden associated with chlorinated and aromatic hydrocarbon solvents.2 CPME and 2-MeTHF are particularly notable because both are derived in part from renewable feedstocks, exhibit low peroxide formation tendency relative to conventional ethers, and have favorable environmental, health and safety (EHS) profiles, attributes that have also led to their adoption in green solid-phase synthesis and liquid-liquid extraction applications beyond chromatography.3 Carbonate esters, including dimethyl carbonate, diethyl carbonate, and propylene carbonate, represent another promising solvent class for both reversed-phase and hydrophilic interaction liquid chromatography (HILIC). Recent joint academic investigations comparing these carbonate esters with classical acetonitrile-based mobile phases across RPLC, HILIC, and normal-phase separations of model compounds found that, with appropriate use of ternary phase diagrams and additives such as tetrabutylammonium perchlorate to manage miscibility, carbonate esters can provide stable, single-phase mobile phases with distinct but controllable selectivity differences relative to acetonitrile.4 The general finding across this body of work is that no single green solvent is a universal drop-in replacement; rather, solvent choice must be tailored to analyte polarity, detection wavelength, and the specific chromatographic mode in use.

Ionic liquids have also been explored as green mobile phase additives, particularly for basic or chelating analytes that suffer from peak tailing on conventional silica-based columns. Imidazolium-based ionic liquid modifiers have been used to develop aqueous mobile phase systems for compounds that are otherwise difficult to elute with acceptable peak shape, illustrating an alternative strategy in which the

aqueous mobile phase itself is reformulated rather than the organic modifier alone.5

2. UHPLC, Column Technology, and Instrumental Miniaturization

Beyond solvent substitution, a second major route to greener HPLC lies in reducing the absolute volume of mobile phase and the time and energy required per analysis. Ultra-high-performance liquid chromatography (UHPLC), employing sub-2 micron fully porous particles or superficially porous particles (core-shell particles) in shorter columns, achieves comparable or superior chromatographic resolution to conventional HPLC in a fraction of the analysis time and solvent volume.6 Quantitative comparisons illustrate the scale of these savings. Scaling an isocratic method from a conventional 150 × 4.6 mm column packed with 5 micron particles down to a 100 × 3.0 mm column with 3 micron particles, or further to a 50 × 3.0 mm column with 1.7 micron particles, has been shown to maintain separation performance while achieving solvent savings of approximately 71.6% and 85.7%, energy reductions of 56.8% and 85.1%, and run-time decreases of 60.2% and 88.5%, respectively, relative to the original method.7 Similarly, transitioning from a 5-micron fully porous particle column to a 1.7-micron UHPLC column for the same separation has been reported to reduce solvent consumption by approximately 85% while cutting analysis time from roughly 30 minutes to under 5 minutes.8 Superficially porous particles offer a further efficiency gain: a 5-micron superficially porous particle column can reduce solvent usage by more than 50% relative to a fully porous particle column of the same nominal size, owing to improved mass transfer kinetics and a flatter van Deemter curve that permits higher flow rates without loss of resolution.8 Column miniaturization extends further into capillary and nano-LC formats. Capillary columns operated at low-to-sub-microliter-per-minute flow rates, when paired with syringe-pump-based miniaturized instrumentation, can reduce mobile phase consumption to approximately 0.1–1% of a conventional HPLC separation using standard 4.6 mm internal diameter columns, and to roughly 1–5% of a typical UHPLC separation using 2.1 mm columns.9 While such ultra-miniaturized formats are most established in proteomics and trace-level applications, they illustrate the technical ceiling of solvent reduction achievable through column and flow-path engineering alone. Monolithic columns, which combine high permeability with efficient mass transfer, similarly allow shorter column lengths and faster analyses, further reducing solvent and energy demand per sample.6 It is important to note that these instrumental gains in greenness are not without trade-offs. UHPLC systems require higher capital investment, generate higher backpressures that demand more robust pumping hardware, and may necessitate re-validation of legacy compendial methods when transferred from conventional HPLC platforms. Nonetheless, the convergence of reduced solvent consumption, reduced energy demand, and reduced analysis time positions UHPLC and related miniaturization strategies among the most impactful and broadly applicable green chromatography interventions identified to date.

Table 1: Comparative Solvent Savings With Column Miniaturization Strategies

Sr. No.	Column Configuration	Particle Size	Approx. Solvent Savings	Approx. Time Savings
1	150 x 4.6 mm (reference)	5 µm	— (baseline)	— (baseline)
2	100 x 3.0 mm	3 µm	~71.6%	~60.2%
3	50 x 3.0 mm	1.7 µm (UHPLC)	~85.7%	~88.5%
4	Capillary LC column	Sub-2 µm / SPP	~99% (vs. 4.6 mm HPLC)	Variable

Data compiled from comparative studies on HPLC-to-UHPLC method scaling.7,8,9

3. Analytical Quality by Design (AQbD) and Rational Green Method Optimization

A recurring inefficiency in conventional HPLC method development is the trial-and-error variation of mobile phase composition, pH, and flow rate, a process that itself consumes considerable solvent and time before a final method is established. Analytical Quality by Design (AQbD), adapted from the Quality by Design framework promoted by the International Council for Harmonisation (ICH), addresses this inefficiency by defining an Analytical Target Profile (ATP) and systematically mapping the relationship between Critical Method Parameters (CMPs) and Critical Method Attributes (CMAs) using statistical Design of Experiments (DoE) rather than one-factor-at-a-time experimentation.10 Recent applications combine AQbD directly with green analytical chemistry assessment. In one representative study, a combined AQbD and green analytical chemistry approach was used to develop an HPLC method for the simultaneous determination of two thalassemia drugs in biological fluid, beginning with a quality risk assessment and a Plackett-Burman screening design across five chromatographic parameters, followed by a custom two-level, three-factor experimental design to identify conditions yielding the highest resolution with acceptable peak symmetry in the shortest run time, thereby minimizing both experimental iterations and solvent waste during development itself.11 In another study, a QbD-driven HPLC method for meropenem trihydrate was developed and validated according to ICH Q2(R1) guidelines, with seven different green analytical chemistry tools subsequently applied to confirm a significant reduction in environmental impact relative to pre-existing methods for the same analyte.12 Box-Behnken and central composite experimental designs are commonly employed within this framework to optimize variables such as mobile phase pH, organic modifier ratio, flow rate, and column temperature against multiple simultaneous responses, including retention time, resolution, theoretical plate count, and peak tailing.13,14 Beyond reducing the number of physical trial runs required, AQbD-developed methods tend to be inherently more robust, since the design space explicitly characterizes how the method behaves across a defined range of conditions rather than at a single optimized point, reducing the likelihood of method failure and subsequent re-development, itself a source of avoidable solvent consumption over a method's operational lifetime.15

An emerging refinement integrates AQbD with explicit greenness and "whiteness" scoring as additional response variables within the design space itself, rather than as a post-hoc evaluation performed only after a method has been finalized. A recent integrative framework of this kind applied AQbD principles alongside up-to-date greenness and whiteness assessment tools to develop a sustainable RP-HPLC method for regulated pharmaceutical products, explicitly incorporating column selection, solvent choice, and method runtime as factors influencing both analytical performance and environmental impact within a single optimization exercise.16 This convergence of statistical method optimization with sustainability metrics represents one of the more methodologically mature directions in the green HPLC literature.

4. Quantitative Greenness Assessment Tools

A defining feature of mature green analytical chemistry practice is the use of standardized, semi-quantitative tools to benchmark and communicate the environmental profile of a method, rather than relying on qualitative claims of "greenness." At least fifteen distinct greenness metrics have been described in the literature, including the National Environmental Methods Index (NEMI), Advanced NEMI, the Analytical Eco-Scale (AES), the Green Analytical Procedure Index (GAPI) and its extended ComplexGAPI variant, the Analytical GREEnness calculator (AGREE) and its sample-preparation-focused counterpart AGREEprep, the HPLC Environmental Assessment Tool (HPLC-EAT), the Analytical Method Volume Intensity (AMVI) index, the Analytical Method Greenness Score (AMGS), and the Blue Applicability Grade Index (BAGI), among others.17 NEMI, among the earliest tools, uses a simple pictogram divided into four quadrants addressing persistent, bioaccumulative and toxic (PBT) reagents, hazardous reagents, corrosive conditions, and waste generation, but offers limited granularity for comparing methods of similar overall profile.17 The Analytical Eco-Scale assigns penalty points based on reagent quantity, hazard classification, energy consumption, and occupational exposure, producing a single numerical score, though the resulting total score does not always indicate which specific step is responsible for a poor rating,

limiting its diagnostic utility for method redesign.18 GAPI, introduced by Płotka-Wasylka in 2018, evaluates the entire analytical workflow from sample collection through final determination using a pictogram composed of a central pentagon surrounded by four additional pentagons, each subdivided into color-coded segments (green, yellow, red) representing low, medium, and high environmental impact at each stage of the procedure.19 This visual format allows rapid identification of the specific steps contributing most to a method's environmental burden, making it particularly useful for guiding targeted greening efforts rather than only reporting an aggregate score.19 AGREE, proposed by Peña-Pereira, Wojnowski and Tobiszewski in 2020, is structured explicitly around the twelve principles of green analytical chemistry, transforming each criterion onto a unified 0–1 scale and generating both a final aggregate score and a visual clock-face pictogram showing performance against each individual principle, with user-adjustable weighting to reflect the relative priorities of a given laboratory or application.20 Because AGREE evaluates each of the twelve principles independently and presents them transparently, it has become one of the most widely adopted tools for new method publications, often reported alongside GAPI or the Eco-Scale for cross-validation of the greenness assessment.20,21 More recent extensions reflect growing analytical sophistication: ComplexGAPI incorporates processes that precede the analytical procedure itself, such as the synthesis of materials used in sample extraction, while AGREEprep applies the AGREE logic specifically to sample preparation workflows.21 The proliferation of these tools, now numbering well over a dozen distinct metrics, has itself become a subject of methodological discussion, with recent comprehensive reviews noting that Eco-Scale, AGREE, NEMI, and GAPI remain the four most widely utilized tools in the published literature, and recommending that authors report at least two complementary metrics, typically one pictogram-based tool such as GAPI and one numerical scoring tool such as AGREE or the Eco-Scale, to provide both diagnostic detail and an easily comparable summary score.17,22

Table 2: Summary of Major Greenness Assessment Tools Used In Green Hplc

Sr. No.	Tool	Year Introduced	Output Format	Primary Strength
1	NEMI	2008 (approx.)	Four-quadrant pictogram	Simplicity, quick visual screen
2	Analytical Eco-Scale (AES)	2012 (approx.)	Single penalty-point score	Widely recognized, easy to compute
3	GAPI / ComplexGAPI	2018	Five-pentagon color pictogram	Whole-workflow, step-by-step diagnosis
4	AGREE / AGREEprep	2020	0–1 score with clock pictogram	Based directly on 12 GAC principles; adjustable weighting
5	HPLC-EAT, AMVI, AMGS, BAGI	2017–2022	Numerical / composite scores	Technique- or stage-specific focus

Compiled from comprehensive reviews of green analytical chemistry metrics.17,18,19,20,21,22

5. Applications in Pharmaceutical, Food, and Environmental Analysis

Pharmaceutical quality control represents the largest application domain for green HPLC, given the sheer volume of routine assay, dissolution, and stability-indicating methods run in regulated environments. A recent comprehensive review on green liquid chromatography for pharmaceutical analysis surveyed published methods spanning HPLC, TLC, UPLC, GC, GC-MS, LC-MS/MS, and HPTLC for impurity profiling published between January 2019 and December 2024, applying greenness assessment frameworks including Eco-Scale, GAPI, NEMI, and AGREE to benchmark reported methods and finding ethanol and dimethyl carbonate to be among the most effective acetonitrile replacements for non-polar to moderately polar pharmaceutical mixtures without compromising chromatographic performance.23 A response-surface-optimized eco-friendly HPLC method for a combination of three antidiabetic drugs,

metformin, glimepiride, and pioglitazone, achieved acceptable linearity and accuracy using an ethanol-modified phosphate buffer mobile phase, with greenness confirmed using both AGREE and GAPI assessments.24 Food analysis applications similarly leverage green HPLC principles for the determination of bioactive compounds, contaminants, and residues in support of clean-label and sustainability claims. A recent comprehensive review of green innovations in HPLC for sustainable food analysis examined eco-friendly solvent systems, miniaturized instrumentation, and greener sample preparation techniques, while also situating these analytical advances within broader regulatory and certification frameworks such as ISO 14001 and ISO 22000.25 High-performance thin-layer chromatography, a related planar separation technique, has undergone a parallel transition, with recent work integrating green, blue, and white analytical chemistry frameworks alongside miniaturized and solvent-reduced sample preparation methods such as QuEChERS, ultrasound-assisted extraction, and dispersive liquid-liquid microextraction.26 Environmental monitoring applications of green HPLC include the determination of pesticide and herbicide residues, pharmaceutical contaminants in wastewater, and organic pollutants in surface water, where the dual goals of method sensitivity and reduced environmental footprint are particularly aligned, since methods that minimize solvent waste in the analytical laboratory also reduce the indirect environmental burden of disposing of waste generated while monitoring environmental contamination elsewhere.27

6. Challenges in Green HPLC Method Development

Despite substantial progress, several practical and scientific challenges continue to limit the wholesale replacement of conventional HPLC methods with green alternatives. First, many green solvents, including ethanol and the carbonate esters, exhibit higher viscosity than acetonitrile, which increases system backpressure and can necessitate either lower flow rates, reduced column length, or instrumentation capable of withstanding higher operating pressures, complicating direct method transfer.1,4

Second, UV cut-off wavelengths differ meaningfully between green and conventional solvents; ethanol, for example, has a higher UV cut-off than acetonitrile, which can introduce baseline drift during gradient elution and limit detection sensitivity at low wavelengths for analytes lacking strong chromophores.1,28 Where such analytes must be detected at low UV wavelengths, the choice of green solvent becomes constrained, sometimes requiring a compromise between full solvent greenness and adequate detection sensitivity.

Third, regulatory and compendial inertia remains a significant barrier. Pharmacopoeial monographs typically specify validated methods using conventional solvents, and replacing an established compendial method with a greener alternative requires a full revalidation exercise, including demonstration of equivalence in accuracy, precision, specificity, and robustness, a process that itself consumes resources and that many laboratories are reluctant to undertake for methods that are already functioning adequately under current guidelines.16,29

Fourth, the proliferation of greenness metrics, now exceeding a dozen distinct tools, has created inconsistency in how greenness is reported across the literature, making direct comparison between methods difficult when different studies apply different combinations of metrics with different weighting schemes.17,22 There is, as yet, no single internationally harmonized standard specifying which metric or combination of metrics should be used for regulatory submission or journal publication, although AGREE and GAPI have emerged as the most commonly co-reported pair.

Fifth, instrumental and capital cost considerations affect the feasibility of UHPLC-based greening strategies in particular; while UHPLC instrumentation delivers substantial solvent and time savings, the higher purchase and maintenance costs of UHPLC systems relative to conventional HPLC instruments can be prohibitive for laboratories in resource-limited settings, partially offsetting the environmental gains with an economic barrier to adoption.6,9

Finally, a genuine methodological tension exists between greenness and analytical performance in

certain applications: not every conventional solvent or technique has an equally effective green substitute for every analyte class, and in some cases, a fully green method may require longer run times, reduced resolution, or narrower linearity ranges relative to the conventional method it replaces, meaning that greenness optimization cannot be pursued in isolation from the fundamental validation criteria that determine whether a method is fit for its intended purpose.16,29

7. Emerging Tools and Future Perspectives

Several converging trends suggest where green HPLC method development is heading over the coming years. The first is the increasing use of computational and artificial intelligence-assisted tools to reduce the number of physical experimental trials required during method development. Predictive retention modelling and machine learning-assisted optimization of chromatographic conditions, layered onto existing AQbD and DoE frameworks, promise to further reduce solvent consumption during the development phase itself by allowing more of the optimization process to occur in silico before physical confirmatory runs are performed, complementing the column and solvent-based advances described above.6,16

The second is the consolidation of green, blue, and white analytical chemistry into integrated assessment frameworks. Where green analytical chemistry addresses environmental and toxicological impact, blue analytical chemistry, formalized through the Blue Applicability Grade Index (BAGI), addresses the practicality, cost, and applicability of a method in real laboratory settings, and white analytical chemistry combines both with traditional analytical performance criteria such as accuracy, precision, and sensitivity into a single holistic evaluation.16,26 This convergence reflects a maturing recognition within the field that a method optimized for greenness alone, at the expense of practicality or analytical reliability, is not genuinely sustainable in a laboratory operations sense, and that true sustainability requires balancing all three dimensions simultaneously.

Third, continued refinement of column technology, including further development of superficially porous particles, monolithic phases, and capillary and chip-based miniaturized formats, is expected to push solvent and energy savings further, particularly as instrument manufacturers increasingly design UHPLC and capillary-flow systems with sustainability as an explicit design goal rather than an incidental benefit of speed-focused engineering.6,9

Fourth, expansion of green solvent libraries beyond the currently dominant set of ethanol, carbonate esters, and bio-derived ethers is likely, particularly for niche applications such as chiral separations, hydrophilic interaction chromatography of highly polar metabolites, and ion-pairing methods, where green substitutes remain comparatively underdeveloped relative to conventional reversed-phase separations of small, moderately polar pharmaceutical molecules.4,28

Finally, harmonization of greenness reporting standards, potentially through coordinated guidance from pharmacopoeial or regulatory bodies analogous to ICH Q2(R2) for method validation, would address the current inconsistency in metric selection and reporting, allowing greenness to be evaluated with the same rigor and comparability currently applied to accuracy, precision, and robustness in conventional method validation.17,22,29 Continued digital automation of greenness calculators, several of which are already available as free downloadable software or web-based tools, is likely to further lower the barrier to routine greenness reporting alongside conventional validation data in future publications.17

CONCLUSION

Green HPLC method development has progressed from a niche research interest into an increasingly mainstream expectation within pharmaceutical and analytical quality control. The strategies reviewed here, namely the substitution of hazardous mobile phase solvents with greener alternatives such as ethanol, carbonate esters, and bio-derived ethers, the adoption of UHPLC and miniaturized column formats to reduce solvent and energy consumption, the integration of Analytical Quality by Design with explicit greenness criteria to rationalize method optimization, and the use of standardized assessment tools such as AGREE, GAPI, and the Analytical Eco-Scale to quantify and communicate sustainability, collectively demonstrate that meaningful reductions

in the environmental footprint of liquid chromatography are achievable without sacrificing analytical performance. Persistent challenges, including solvent viscosity and UV cut-off limitations, regulatory and compendial inertia, inconsistent greenness metric reporting, and instrumentation cost, continue to temper the pace of adoption, particularly in resource-limited settings. Looking forward, the convergence of artificial intelligence-assisted method optimization, the integrated green-blue-white analytical chemistry framework, and continued advances in column and solvent technology suggest that green HPLC will continue to mature into a more standardized and widely practiced discipline. Sustained progress will depend on harmonized greenness reporting standards and continued collaboration between method developers, regulators, and instrument manufacturers to ensure that environmental responsibility and analytical rigor advance together rather than as competing priorities.

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