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Review Article

Advances in Green HPLC for Pharmaceutical Analysis: Method Development, Validation, and Sustainability Assessment

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ABSTRACT

High-performance liquid chromatography (HPLC) remains one of the most important analytical platforms in pharmaceutical quality control, stability testing, impurity profiling, dissolution testing and bioanalytical support. However, conventional HPLC workflows may consume substantial volumes of organic solvents, generate hazardous waste, require energy-intensive operation and depend on sample-preparation steps that increase the environmental footprint of analysis. Green analytical chemistry (GAC) therefore seeks to redesign analytical procedures so that analytical performance is retained while resource use, hazard, waste and energy demand are minimized. This review examines the development of greener HPLC methods for pharmaceutical analysis with emphasis on solvent selection, aqueous-rich mobile phases, ethanol and other preferable solvents, reduced flow rates, short and narrow-bore columns, high-efficiency stationary phases, direct analysis, miniaturized sample preparation, automation, and analytical quality by design (AQbD). The review further discusses validation in the context of ICH Q2 (R2), including specificity, accuracy, precision, range, detection and quantitation limits, robustness and system suitability, and explains why greenness should be treated as a design constraint rather than a post-development decoration. Major assessment approaches—including NEMI, Analytical Eco-Scale, GAPI, HPLC-EAT, AGREE and related multidimensional tools—are compared with respect to scope, interpretability, strengths and limitations. A practical framework is proposed for combining chromatographic performance, regulatory validation and sustainability metrics into a single method-selection strategy. Recent literature shows a transition from simply replacing acetonitrile or methanol toward broader sustainability optimization involving solvent volume, energy, sample preparation, instrument

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utilization, waste treatment, and life-cycle thinking. The review concludes that the next generation of pharmaceutical HPLC will increasingly combine AQbD, automation, miniaturization, safer solvents, digital optimization and circular analytical practices. Such integration can support reliable pharmaceutical quality assessment while reducing occupational and environmental burden.

INTRODUCTION

Pharmaceutical analytical chemistry has a dual responsibility: it must generate scientifically defensible measurements and support a manufacturing system that is safe, efficient and increasingly sustainable. HPLC occupies a central position because of its selectivity, reproducibility, broad applicability and compatibility with ultraviolet, fluorescence, mass-spectrometric and other detectors. It is routinely applied to assay active pharmaceutical ingredients (APIs), quantify related substances and degradation products, support dissolution testing, characterize formulations, and investigate stability. The analytical laboratory, however, can consume large quantities of solvents and disposable materials relative to the amount of analyte ultimately measured.

The sustainability challenge is particularly important for reversed-phase HPLC, where acetonitrile and methanol are commonly used as organic modifiers and where conventional analytical methods may operate at millilitre-per-minute flow rates for many minutes. A single sequence containing hundreds of injections can therefore generate litres of mobile-phase waste. In addition to solvent volume, the environmental profile depends on solvent toxicity, volatility, production pathway, waste treatment, energy use, sample

preparation, instrument utilization and the fate of consumables. Green analytical chemistry extends the concept of green chemistry into measurement science by emphasizing prevention of waste, reduction of hazardous substances, miniaturization, automation, direct analysis and energy efficiency.

The 12 principles of green analytical chemistry proposed by Galuszka and co-workers provide a practical framework for designing analytical methods with reduced sample and reagent requirements, fewer operations, lower waste and improved safety. These principles complement rather than replace the original principles of green chemistry.

The recent literature demonstrates that green liquid chromatography has moved beyond simple solvent substitution. Contemporary approaches integrate greener solvent systems with short columns, core-shell or sub-2- μm particles, lower flow rates, reduced injection volumes, direct sample preparation, automated workflows and systematic greenness evaluation. A 2024 review of green LC for pharmaceutical analysis highlighted NEMI, Analytical Eco-Scale, GAPI, AMGS and AGREE as important tools and discussed the emerging relationship between green analytical chemistry, white analytical chemistry and circular analytical chemistry.

This review therefore focuses specifically on the pharmaceutical HPLC workflow, from analytical target definition and method development to validation and sustainability assessment. Particular attention is given to practical decisions that can be implemented in academic laboratories and pharmaceutical quality-control environments.



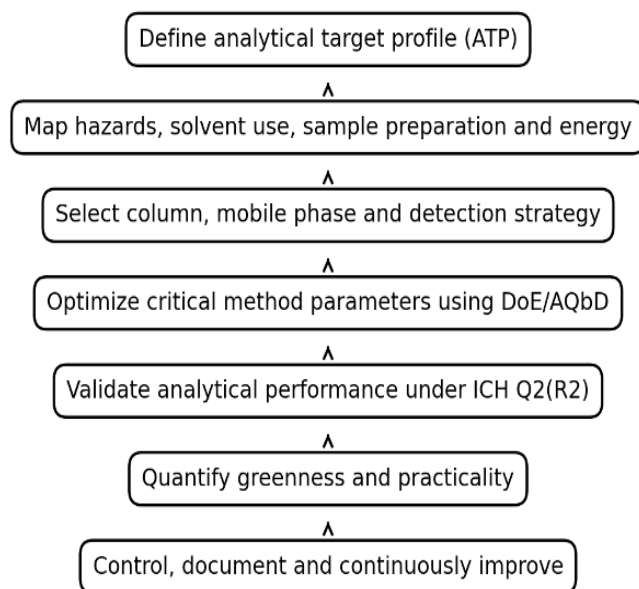


Figure 1: Green HPLC Method-Development Workflow Proposed for Pharmaceutical Analysis.

GREEN ANALYTICAL CHEMISTRY AND THE PHARMACEUTICAL HPLC CONTEXT

Green analytical chemistry is concerned with reducing the environmental and health burden of analytical measurements without compromising the information quality required for a scientifically valid decision. In pharmaceutical analysis, this balance is especially important because analytical methods support decisions about identity, strength, purity, stability and product release. A method that is environmentally benign but insufficiently selective or precise is not an acceptable pharmaceutical method.

Twelve principles of green analytical chemistry

The GAC principles emphasize direct measurement, minimal sample size, in-situ analysis when possible, integration of analytical operations, automation and miniaturization, avoidance of derivatization, waste prevention, renewable or safer reagents, elimination of hazardous substances, longer instrument lifetime and increased sample throughput. Their practical value is that they translate sustainability from an abstract objective into design criteria that can be considered before a method is finalized.

GAC principle	Implication for HPLC	Typical implementation
Direct analytical techniques	Avoid unnecessary extraction/derivatization	Direct dilution and injection when matrix permit
Minimal sample and solvent	Reduce material intensity	Lower injection volume and sample concentration
Integration	Reduce separate operations	Online dilution, automated preparation
Miniaturization	Reduce solvent and energy	Narrow-bore/short columns, low-flow LC
Avoid derivatization	Reduce reagents and waste	Direct UV/PDA or MS-compatible detection
Waste prevention	Minimize generated waste	Shorter run time, lower flow, smaller reservoirs
Safer chemicals	Reduce hazard	Ethanol, water and other preferable solvents where suitable
Energy efficiency	Reduce power demand	Shorter runs, efficient detectors and instrument standby
Automation	Improve reproducibility and reduce handling	Autosamplers and integrated workflows



GAC principle	Implication for HPLC	Typical implementation
Renewable resources	Prefer renewable feedstocks where technically valid	Bio-derived solvent options when validated
Lifecycle thinking	Consider production and disposal	Solvent recovery and waste segregation
Analytical quality	Maintain reliable information	Validation, robustness and system suitability

WHY CONVENTIONAL HPLC NEEDS SUSTAINABILITY OPTIMIZATION

The environmental burden of an HPLC method is not determined by the solvent name alone. Two methods using the same solvent can have substantially different

footprints if one uses 1.0 mL/min for 20 min and the other uses 0.3 mL/min for 5 min. Likewise, a method that replaces acetonitrile with ethanol but requires very high pressure, longer retention, more injections or repeated sample extraction may not represent a net sustainability improvement.

Parameter	Conventional tendency	Greener target
Organic solvent	High proportion of acetonitrile/methanol	Aqueous-rich or preferable solvent system
Flow rate	Often 0.8–1.5 mL/min in 4.6-mm columns	Use the lowest validated flow compatible with performance
Run time	Long isocratic/gradient cycles	Short, high-efficiency separation
Column	Long conventional column	Short/narrow-bore or high-efficiency column
Sample preparation	Multiple extraction/filtration steps	Direct dilution or miniaturized preparation
Waste	High solvent and vial consumption	Low-volume injections and reduced sequence waste
Energy	Long instrument operation	Shorter runs and efficient thermal control
Method optimization	Trial-and-error	AQbD/DoE-guided development
Sustainability evaluation	Often absent	Routine use of green metrics

GREEN SOLVENTS AND MOBILE-PHASE DESIGN

Solvent choice is among the most visible decisions in green HPLC. Acetonitrile provides favorable chromatographic properties and low viscosity, but its toxicity and life-cycle considerations encourage evaluation of alternatives when chromatographic performance allows. Methanol is widely used and is often effective, but it is also hazardous and can generate significant waste. Ethanol is attractive because of its comparatively favorable safety profile and availability from renewable feedstocks in some supply chains; however,

its higher viscosity can increase back pressure and may require adjustments in flow rate, temperature, column dimensions or gradient design.

Other solvents such as isopropanol, ethyl acetate and selected bio-based solvents may be useful for particular separations. Their suitability must be judged using chromatographic selectivity, UV transparency, viscosity, miscibility, pressure, analyte stability and compatibility with the detector. A solvent should not be labelled 'green' solely because it is bio-derived; the entire workflow and solvent life cycle should be considered.

Solvent / system	Potential advantage	Key limitation in HPLC	When to consider
Water	Very low hazard; inexpensive	Limited elution strength for hydrophobic analytes	Aqueous-rich RP-HPLC
Ethanol	Preferable safety profile; renewable routes possible	Higher viscosity and pressure than ACN	RP-HPLC when selectivity permits
Methanol	Widely available; good chromatographic utility	Toxicity and waste burden	When needed for selectivity or detector compatibility



Solvent / system	Potential advantage	Key limitation in HPLC	When to consider
Acetonitrile	Low viscosity; strong and versatile eluent	Toxicity and supply/life-cycle concerns	When performance benefit clearly outweighs alternatives
Isopropanol	Useful for selectivity and strong elution	High viscosity	Specialized gradients or normal-phase/SEC contexts
Ethyl acetate	Relatively preferable in some applications	Limited water miscibility; UV/phase constraints	Specific extraction/normal-phase applications
Bio-based solvents	Potential lower fossil dependence	Variable toxicity, availability and chromatographic behavior	After performance and life-cycle screening

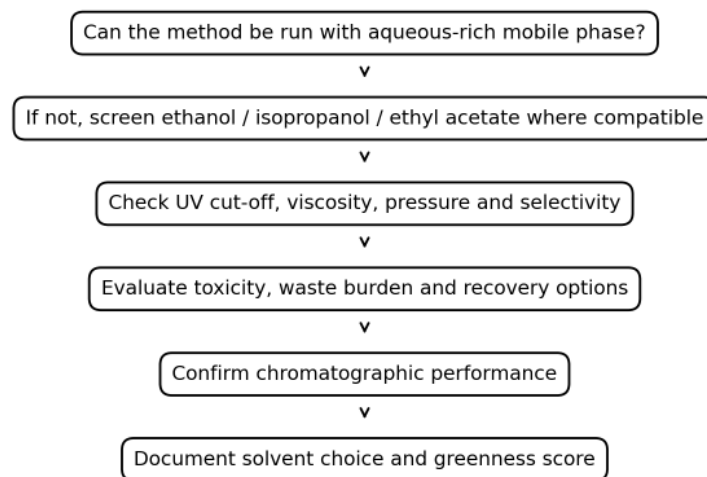


Figure 4: Practical Decision Pathway for Selecting a Greener Mobile-Phase Solvent.

GREEN HPLC METHOD DEVELOPMENT

Green method development should begin before the first chromatographic experiment. The analytical target profile (ATP) should define the intended measurement, matrix, analyte concentration range, required selectivity, expected impurities, detection mode and decision criteria. Sustainability objectives can then be added to the ATP, such as maximum mobile-phase volume per injection, preferred solvent class, maximum run time, target energy use and avoidance of unnecessary sample preparation.

Analytical target profile and critical method attributes

- Define the intended use: assay, impurities, dissolution, content uniformity, stability, cleaning verification or another purpose.
- Identify critical quality attributes of the measurement: resolution, accuracy, precision, sensitivity, selectivity and reporting range.

- Identify critical method parameters: organic fraction, pH, buffer concentration, column chemistry, temperature, flow rate, injection volume and gradient profile.
- Add sustainability criteria: solvent hazard, solvent volume, waste, energy demand, number of sample-preparation steps and potential for solvent recovery.
- Set system-suitability criteria before optimization so that green improvements do not become detached from analytical quality.

Column selection and miniaturization

Column dimensions are powerful sustainability levers because solvent consumption is directly related to flow rate and run duration. Short columns can reduce analysis time, while narrow-bore columns can reduce volumetric flow. Core-shell particles can provide high efficiency at lower back pressure than some fully porous sub-2- μm systems, depending on column dimensions and instrument capabilities. The choice should



nevertheless be validated for resolution, robustness and pressure stability.

Flow-rate and run-time optimization

Reducing flow rate reduces solvent consumption per unit time, but an excessively low flow may broaden peaks, reduce throughput and increase total sequence time. The relevant sustainability variable is therefore not simply flow rate but solvent volume per analysis. A useful design equation is: mobile-phase volume per injection (mL) = flow rate (mL/min) × run time (min). This simple calculation should become a routine development metric.

Sample preparation

Sample preparation can dominate the footprint of an analytical workflow. Liquid-liquid extraction, repeated centrifugation, evaporation, derivatization and extensive filtration add solvents, plastics and energy consumption. Where matrix and sensitivity allow, direct dilution, dilute-and-shoot, microextraction, solid-phase microextraction, micro-solid-phase extraction and other miniaturized approaches can substantially reduce material use. The final choice must be driven by analyte

recovery, matrix effects and reproducibility rather than greenness alone.

Detection strategy

PDA/UV detection can be attractive for routine pharmaceutical assays when analyte absorptivity and selectivity are sufficient. Fluorescence or MS may provide greater sensitivity and selectivity but may involve additional gases, solvents or energy demand. Detector selection should therefore be made according to the analytical objective, with the simplest adequate detection principle generally preferred.

AQbD and design of experiments

AQbD provides a structured route for reducing empirical trial-and-error. Instead of changing one variable at a time, a statistically designed experiment can model the effects of mobile-phase composition, pH, flow rate, temperature and other factors on chromatographic responses. Sustainability responses can be incorporated alongside analytical responses. For example, the optimization function can simultaneously maximize resolution and plate count, minimize tailing and run time, and minimize solvent volume.

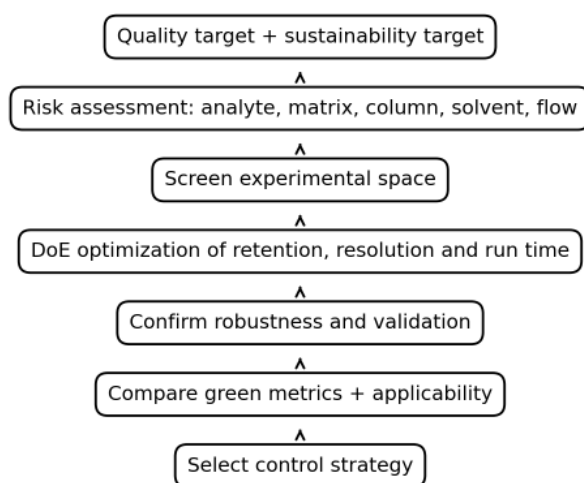


Figure 2: Integration of Analytical Quality by Design (AQbD) with Sustainability Objectives.

VALIDATION OF GREEN HPLC METHODS

A green analytical method must satisfy the same fundamental expectations of analytical reliability as any other pharmaceutical method. ICH Q2 (R2), effective in the European Union from 14 June 2024, provides the current framework for validation of analytical

procedures used for purposes such as assay, purity, impurities and other quantitative or qualitative measurements.

Validation should be proportionate to the analytical objective. ICH Q2 (R2) emphasizes selecting validation characteristics according to the purpose of the

procedure, with specificity, range, accuracy and precision forming central performance characteristics for many quantitative methods.

Validation characteristic	Purpose	Typical green-HPLC considerations
Specificity/selectivity	Demonstrate measurement of analyte without relevant interference	Ensure solvent substitution or shortened separation does not compromise impurity resolution
Linearity/response model	Establish relationship across intended range	Use minimum practical concentration points while retaining statistical adequacy
Range	Define validated measurement interval	Align with intended pharmaceutical specification and sample concentration
Accuracy	Assess closeness to true/accepted value	Recovery experiments should use minimal material consistent with design
Repeatability	Assess precision under same conditions	Optimize injection sequence without unnecessary replicate waste
Intermediate precision	Assess analyst/day/instrument variation	Use a rational design rather than excessive experiments
LOD	Establish detection capability where applicable	Miniaturization may improve sensitivity but should not be assumed to
LOQ	Establish quantitative capability where applicable	Confirm precision and accuracy at LOQ
Robustness	Evaluate sensitivity to deliberate small changes	Include environmentally relevant parameters such as solvent composition and flow
System suitability	Verify ongoing chromatographic performance	Use performance criteria that protect resolution while avoiding unnecessary injections

Specificity

Specificity is especially important when green method development uses shorter columns, faster gradients or alternative solvents. A reduction in analysis time can unintentionally reduce separation between the API and degradation products. Specificity should therefore be demonstrated using appropriate blank, placebo, standard, sample and stressed or spiked samples as applicable to the method objective.

Accuracy and precision

Accuracy and precision should be demonstrated across the validated range using scientifically justified replicate levels. A greener method should not be granted relaxed acceptance criteria merely because it is environmentally preferable. Instead, the development strategy should seek equivalent or improved analytical performance with lower resource use.

Robustness

Robustness has a sustainability dimension because a method that fails under minor operating variation

generates repeat injections, repeat sample preparation and additional waste. Robustness studies should therefore consider variables that are both chromatographically critical and environmentally relevant, including flow rate, organic fraction, pH, temperature and column lot where appropriate.

SUSTAINABILITY ASSESSMENT OF HPLC METHODS

Greenness assessment converts sustainability concepts into a structured comparison. No single metric captures every dimension of an analytical method, so multiple complementary tools may be needed. NEMI provides a simple pictorial screen, whereas Analytical Eco-Scale applies penalty points. GAPI evaluates the full workflow from sample collection to determination. HPLC-EAT was developed specifically to profile environmental, health and safety impacts of LC solvents. AGREE provides a 0–1 score based on the 12 GAC principles and an interpretable pictogram.

Tool	Main output	Major strength	Important limitation	Best use
NEMI	Four-quadrant pictogram	Very simple and rapid	Limited granularity	Initial screening



Tool	Main output	Major strength	Important limitation	Best use
Analytical Eco-Scale	Penalty-point score (100 ideal)	Quantitative and easy to compare	Penalty selection can influence result	Routine method comparison
GAPI	Five-pentagram pictogram	Covers whole analytical procedure	Semi-quantitative	Visual workflow diagnosis
HPLC-EAT	LC-specific environmental/H&S profile	Designed for HPLC solvent assessment	Less comprehensive for all lifecycle dimensions	LC solvent comparison
AGREE	0–1 greenness score + pictogram	Integrates 12 GAC principles	Requires informed parameter input	Overall GAC assessment
AGREEprep	Preparation-focused extension	Highlights sample-preparation burden	Requires appropriate preparation data	Methods with significant extraction
AMGS / related scores	Composite score	Useful for benchmarking	Different tools may weight criteria differently	Comparative research
Life-cycle assessment	Environmental impacts across life cycle	Most comprehensive conceptual scope	Data intensive	High-value sustainability studies

NEMI

The National Environmental Methods Index approach classifies methods using four binary criteria related to persistent, bioaccumulative or toxic chemicals, hazardous reagents, corrosive reagents and waste generation. Its simplicity is useful for rapid comparison, but it can overlook meaningful differences in solvent volume, energy use and sample-preparation intensity.

Analytical Eco-Scale

Analytical Eco-Scale begins with an ideal score of 100 and subtracts penalty points associated with reagent hazards, quantities, energy, waste and other undesirable features. Scores above 75 are commonly interpreted as excellent greenness, 50–75 as acceptable and below 50 as inadequate, although these categories should be interpreted within the context of the method and study design.

GAPI

GAPI provides a visual assessment of the entire analytical procedure. Its pentagram-style pictogram uses

color zones to identify low, medium and high environmental impact at different stages. This is particularly useful for identifying whether the main sustainability problem lies in sampling, preparation, reagents or instrumental determination.

HPLC-EAT

HPLC-EAT was developed specifically for HPLC and considers environmental, health and safety issues associated with solvents. It is therefore useful when solvent selection is the main development question. The tool can complement broader metrics such as AGREE or GAPI rather than replace them.

AGREE

AGREE translates the 12 GAC principles into a unified 0–1 scale and presents the outcome as a pictogram. It considers multiple dimensions, including sample size, solvent use, energy, waste and other procedural characteristics. Its major advantage is that it provides a single interpretable score while retaining visual information about individual principles.

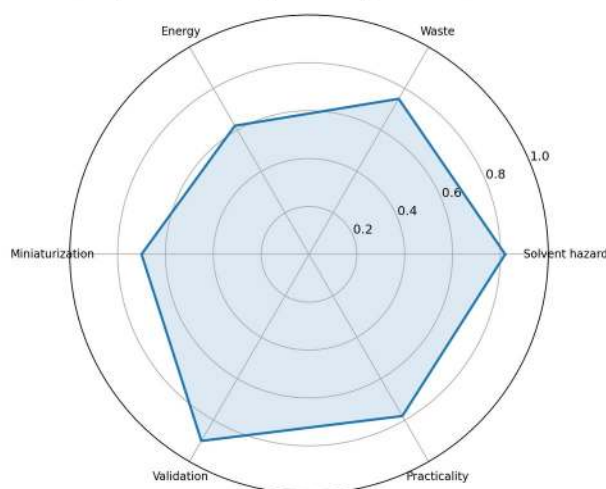


Figure 3: Conceptual Sustainability Profile Illustrating How a Method Can Be Evaluated Across Multiple Dimensions. the Figure Is Illustrative, Not a Numerical Assessment of a Specific Published Method.

RECENT DEVELOPMENTS AND REPRESENTATIVE LITERATURE

Recent reviews show rapid expansion of green LC applications in pharmaceutical analysis. Ahmed et al. summarized pharmaceutical green LC studies and highlighted the use of GAPI, NEMI, Eco-Scale, AMGS and AGREE, together with emerging concepts such as

white analytical chemistry and circular analytical chemistry. similarly reviewed recent green LC approaches and emphasized the continued use of GAPI, NEMI, Eco-Scale, AMGS and AGREE. A 2026 review has further emphasized the combination of AQbD with green HPLC/UPLC, including solvent reduction, miniaturized sample preparation and quantitative sustainability evaluation.

Development trend	Analytical objective	Sustainability contribution	Development caution
Aqueous-rich RP-HPLC	Routine assay/impurities	Reduces organic solvent demand	May alter selectivity and retention
Ethanol-based RP-HPLC	Replace/reduce less-preferable solvent	Potentially lower hazard/fossil dependence	Higher viscosity and pressure
Short-column LC	Faster analysis	Lower solvent volume per injection	Resolution may decrease
Narrow-bore LC	Lower flow	Lower mobile-phase consumption	Requires suitable instrument and tubing
Core-shell particles	High efficiency	Potentially shorter columns/runs	Pressure and method transfer must be controlled
Direct injection	Reduce sample preparation	Less solvent, plastic and energy	Matrix effects may limit use
Microextraction	Trace/selective analysis	Lower extraction solvent volume	Method complexity may increase
AQbD/DoE	Robust method development	Fewer empirical experiments; optimized resource use	Requires statistical expertise
Automation	Throughput and reproducibility	Less manual handling; standardized consumption	Equipment and maintenance burden
Solvent recovery	Waste management	Potentially reduces disposal and virgin solvent demand	Economic and technical feasibility required

INTEGRATING VALIDATION AND SUSTAINABILITY INTO A SINGLE DEVELOPMENT FRAMEWORK

The principal methodological argument of this review is that analytical quality and greenness should be optimized together. Treating greenness as a final scoring exercise can create a misleading outcome: a method may score well because it uses little solvent while failing to resolve critical impurities. Conversely, a highly robust method may be unnecessarily resource-intensive because solvent volume, run time or sample preparation were never included as development responses.

A practical integrated framework is to define two groups of responses. The first group contains analytical responses such as resolution, theoretical plates, tailing factor, retention time, peak area precision and recovery. The second group contains sustainability responses such as solvent volume per sample, hazardous-solvent fraction, number of preparation steps, waste mass or volume and approximate energy demand. Multi-response desirability or constrained optimization can then identify operating regions that meet analytical acceptance criteria while minimizing environmental burden.

Stage	Analytical decision	Sustainability decision	Output
1. Define ATP	What must be measured?	What resource/hazard limits are desirable?	Combined target profile
2. Risk assessment	Which variables affect performance?	Which variables drive footprint?	Critical method parameters
3. Screening	Identify viable chromatographic space	Reject high-hazard/high-waste options early	Candidate method space
4. Optimization	Maximize resolution/precision	Minimize solvent, run time and waste	Design space
5. Validation	Demonstrate intended performance	Confirm green design is retained	Validated green method
6. Metric assessment	Document quality	Quantify greenness	Sustainability profile
7. Control strategy	Monitor system suitability	Monitor solvent use and waste	Continual improvement

PROPOSED PRACTICAL PROTOCOL FOR GREEN HPLC DEVELOPMENT IN PHARMACEUTICAL LABORATORIES

- Step 1: State the analytical purpose and required reporting decision.
- Step 2: Establish an ATP containing both performance and sustainability objectives.
- Step 3: Prefer direct analysis and minimum sample preparation whenever scientifically justified.
- Step 4: Screen water-rich mobile phases and preferable organic solvents before defaulting to conventional solvent systems.
- Step 5: Compare stationary phases and column dimensions with solvent consumption and pressure as development responses.
- Step 6: Use DoE/AQbD to study the most influential chromatographic parameters rather than relying exclusively on one-factor-at-a-time experimentation.

- Step 7: Minimize run time and flow while preserving resolution and system suitability.
- Step 8: Validate according to the intended use and current ICH Q2 (R2) principles.
- Step 9: Evaluate the finalized method using at least two complementary green metrics, preferably including a broad metric such as AGREE or GAPI and an LC-focused assessment such as HPLC-EAT.
- Step 10: Record solvent volume, waste, energy-related observations and consumables so that the method can be improved over its operational lifetime.

TEMPLATE FOR REPORTING A GREEN HPLC CASE STUDY

For publication-quality reporting, authors should provide enough information for independent sustainability assessment. The following minimum dataset is recommended: analyte and matrix; column dimensions and



stationary phase; mobile-phase composition; solvent grade; flow rate; temperature; injection volume; detection wavelength or detector type; total run time; sample-preparation steps and solvent volumes; number

of injections; waste volume; system-suitability criteria; validation results; and the values or inputs used for each green metric.

Reported item	Minimum information
Chromatography	Column chemistry, dimensions, particle size, temperature, flow
Mobile phase	Solvents, proportions, pH/buffer, gradient program
Injection	Volume and concentration
Detection	Detector type, wavelength/conditions
Sample preparation	All steps, solvent volumes, filtration and extraction
Validation	Specificity, linearity/range, accuracy, precision, LOD/LOQ where relevant, robustness
System suitability	Resolution, plates, tailing, %RSD or other predefined criteria
Sustainability	Solvent volume, waste, metric scores and assumptions
Operational data	Number of samples, sequence duration, instrument utilization
Waste management	Segregation, treatment, recovery or disposal route

REGULATORY AND QUALITY-CONTROL CONSIDERATIONS

Regulatory acceptance is driven primarily by fitness for purpose, validated performance and control of the analytical procedure. Current ICH Q2 (R2) provides a harmonized validation framework, while Q14 addresses analytical procedure development and encourages a science- and risk-based approach. Green objectives should therefore be integrated into development without weakening established expectations for analytical reliability.

In pharmaceutical quality control, method transfer can also be a sustainability issue. A method that depends on a particular instrument configuration, unusually low dwell volume or specialized column may become difficult to transfer, leading to redevelopment and additional solvent consumption. Robust method design should therefore include transferability, instrument availability and lifecycle considerations.

Documentation should distinguish between a method that is validated and a method that has been shown to be greener. Validation demonstrates analytical fitness; greenness assessment characterizes sustainability. Both are needed for a credible green-HPLC claim.

CHALLENGES AND LIMITATIONS

- Green-solvent substitution can change selectivity, viscosity, pressure and detector compatibility.

- Different greenness metrics use different boundaries, assumptions and weighting schemes; scores from different tools are not directly interchangeable.
- Many studies report greenness without providing enough raw information to reproduce the assessment.
- Life-cycle impacts of solvent manufacture, transport, purification and disposal are often unavailable at laboratory level.
- Miniaturization may require specialized instruments or columns and may create a practical barrier in smaller laboratories.
- Shorter methods may increase co-elution risk and can require more sophisticated detectors.
- Solvent recovery is not automatically greener if the recovery process consumes excessive energy or produces secondary waste.
- Regulatory and compendial familiarity can slow adoption of nontraditional solvents or rapidly changing chromatographic platforms.
- Some published claims use the term 'green' without quantitative comparison to a conventional method; such claims should be interpreted cautiously.

FUTURE PERSPECTIVES

The future of sustainable pharmaceutical chromatography is likely to involve convergence rather than a



single technological breakthrough. AQbD can reduce experimental burden; machine-learning and Bayesian optimization can accelerate exploration of chromatographic design spaces; miniaturized LC can reduce solvent use; automated sample preparation can reduce handling; and digital laboratory systems can capture resource-use data for continuous improvement.

White analytical chemistry provides a useful conceptual direction because it considers green, analytical-

performance and practical/economic dimensions together. Circular analytical chemistry extends this further by emphasizing resource circulation, waste minimization and lifecycle thinking. Recent literature places these concepts alongside established green metrics and suggests that sustainability evaluation is moving from a single 'greenness score' toward multidimensional decision frameworks.



Figure 5: Roadmap from Conventional Chromatography Toward Circular and White Analytical Chemistry.

CONCLUSION

Green HPLC represents a transition from solvent substitution toward comprehensive redesign of pharmaceutical analytical workflows. The most effective strategies combine safer solvent selection with reduction of solvent volume, shorter analysis time, miniaturized columns, efficient stationary phases, reduced sample preparation, automation and statistically guided optimization. Importantly, environmental improvement must be achieved without compromising specificity, accuracy, precision, robustness or other requirements of the analytical purpose.

Greenness assessment tools provide complementary information rather than a universal ranking system. NEMI is useful for rapid screening; Eco-Scale offers a penalty-based quantitative view; GAPI provides a whole-workflow visual profile; HPLC-EAT focuses on LC solvent impacts; and AGREE integrates the 12 GAC principles into a unified score. The strongest reporting practice is therefore to combine at least two metrics and disclose all assumptions and raw inputs.

The integration of AQbD, ICH Q2 (R2) validation and sustainability assessment offers a practical framework for pharmaceutical laboratories. Future green HPLC methods should be designed around both analytical target profiles and sustainability targets, with continuous monitoring of solvent consumption, waste and operational efficiency. This approach can move pharmaceutical analysis toward safer, lower-waste and

more resource-efficient laboratory practice while preserving the high level of analytical confidence required for medicine quality.

DECLARATIONS

Author contributions

Sandrapati Keerthana Madhuri: Conceptualization, literature review, manuscript drafting and scientific editing. Dr. M. Kishore Babu: Supervision, critical review and final approval. Ch. Maruthi Manoj, D. Newlin Preethy and Siddha Geetika: Literature collection, data organization, table preparation and manuscript review. All authors approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

No new experimental dataset was generated. Literature sources used for the review are listed in the references.

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