

GREEN ANALYTICAL CHEMISTRY IN MODERN PHARMACEUTICAL QUALITY CONTROL: PRINCIPLES, EMERGING TECHNOLOGIES, INDUSTRIAL IMPLEMENTATION, AND REGULATORY PERSPECTIVES

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ABSTRACT

The pharmaceutical industry is undergoing a paradigm shift toward sustainable manufacturing and environmentally responsible quality assurance, driven by increasing regulatory expectations and global commitments to green chemistry. Green Analytical Chemistry (GAC) has emerged as a transformative approach to pharmaceutical quality control by minimizing the consumption of hazardous chemicals, reducing waste generation, improving energy efficiency, and enhancing analytical performance without compromising data quality or regulatory compliance. This review critically examines the evolving role of GAC in modern pharmaceutical quality control, with particular emphasis on emerging analytical technologies, industrial implementation strategies, regulatory considerations, and future research directions. The review discusses the fundamental principles and metrics of GAC, including Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), Analytical GREENness (AGREE), and complementary assessment tools for evaluating analytical sustainability. Recent advancements in environmentally benign analytical methodologies — including green high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), spectroscopic techniques, miniaturized and microfluidic analytical systems, ambient ionization mass spectrometry, and solvent-free or solvent-reduced sample preparation approaches — are comprehensively evaluated for their applicability in pharmaceutical quality control. Furthermore, the integration of Process Analytical Technology (PAT), Quality by Design (QbD), chemometrics, artificial intelligence (AI), machine learning (ML), automation, and digital analytical platforms is explored as a means of improving method robustness, real-time monitoring, predictive quality assessment, and sustainable decision-making. The review also highlights the opportunities and challenges associated with industrial adoption of GAC, including method validation, lifecycle management, technology transfer, economic feasibility, workforce training, and harmonization with international regulatory expectations. Particular attention is given to current regulatory frameworks established by ICH, the United States Pharmacopeia (USP), the European Pharmacopoeia (Ph. Eur.), and other global agencies, while identifying existing gaps in the standardization and regulatory acceptance of green analytical methodologies. Overall, this review provides a comprehensive and critical perspective on how Green Analytical Chemistry is reshaping pharmaceutical quality control by integrating sustainability with analytical excellence. It underscores the necessity for collaborative efforts among academia, industry, and regulatory authorities to accelerate the implementation of greener analytical practices, thereby supporting environmentally sustainable pharmaceutical development while maintaining the highest standards of product quality, safety, and regulatory compliance.

KEYWORDS: Green Analytical Chemistry (GAC); Pharmaceutical Quality Control; Sustainable Analytical Methods; Green HPLC; UHPLC; Supercritical Fluid Chromatography (SFC); Capillary Electrophoresis (CE); Process Analytical Technology (PAT); Quality by Design (QbD); Artificial Intelligence (AI); Chemometrics; Analytical Eco-Scale; AGREE; Green Analytical Procedure Index (GAPI); Method Validation; Pharmaceutical Analysis.

1. INTRODUCTION

Pharmaceutical quality control (QC) laboratories are among the highest consumers of organic solvents, reagents, and energy in the chemical sciences, generating substantial volumes of hazardous laboratory waste through routine identification, assay, dissolution, related-substance, and stability-indicating testing. Conventional reversed-phase high-performance liquid chromatography (HPLC) methods alone, when scaled across the thousands of batch releases performed daily worldwide, consume enormous quantities of acetonitrile and methanol — solvents that are costly, petroleum-derived, and classified as hazardous to aquatic life and human health. This reality, combined with tightening environmental regulations, corporate sustainability commitments, and the broader global movement toward the United Nations Sustainable Development Goals, has catalysed the emergence of Green Analytical Chemistry (GAC) as a mainstream discipline within pharmaceutical analysis rather than a peripheral academic concern.^[1,6]

Green Analytical Chemistry extends the twelve principles of green chemistry, originally articulated for synthetic organic processes, into the domain of analytical measurement. Its central philosophy is not that greenness should override analytical performance, but that environmentally benign strategies — solvent substitution, miniaturization, automation, direct analysis, and energy reduction — can very often be achieved while maintaining or even improving accuracy, precision, sensitivity, and regulatory compliance.^[5,6] This is a critical distinction for pharmaceutical quality control, where any change in analytical methodology must be validated against the rigorous standards of the International Council for Harmonisation (ICH), the United States Pharmacopeia (USP), and the European Pharmacopoeia (Ph. Eur.) before it can be adopted for batch release testing.

This review presents a comprehensive, M.Pharm-level examination of GAC as applied to pharmaceutical quality control. It begins with the historical evolution and guiding principles of GAC, followed by a detailed treatment of the quantitative and semi-quantitative metrics used to assess method greenness. It then surveys green sample-preparation strategies, green chromatographic and electrophoretic separation techniques, spectroscopic and ambient-ionization approaches, and miniaturized/microfluidic platforms. A dedicated section explores the convergence of GAC with Process Analytical Technology (PAT), Quality by Design (QbD), chemometrics, and artificial intelligence, since these frameworks increasingly determine how greener methods are designed, validated, and deployed at industrial scale. The review closes with a critical discussion of industrial implementation challenges, the current regulatory landscape, and future research priorities.

2. Principles and Philosophy of Green Analytical Chemistry

2.1 Historical Evolution

The conceptual roots of GAC trace back to 1995, when Paul Anastas first articulated the need to extend green chemistry ideology into the analytical sciences in an editorial devoted to miniaturisation, containment, and reagent replacement. Over the subsequent two decades, GAC matured from an informal set of best practices into a structured discipline with codified principles, quantitative assessment tools, and a dedicated body of peer-reviewed literature. Figure 1 traces this evolution, from the earliest reagent-replacement and miniaturisation concepts of the mid-1990s through the National Environmental Methods Index (NEMI), the Analytical Eco-Scale of Gałuszka et al. (2012), the formal twelve-principle GAC framework, the Green Analytical Procedure Index (GAPI) of Płotka-Wasyłka (2018), the Analytical GREENess (AGREE) calculator of Pena-Pereira et al. (2020), and the most recent extensions such as White Analytical Chemistry (WAC), AGREEprep, and ComplexGAPI.^[9,10]

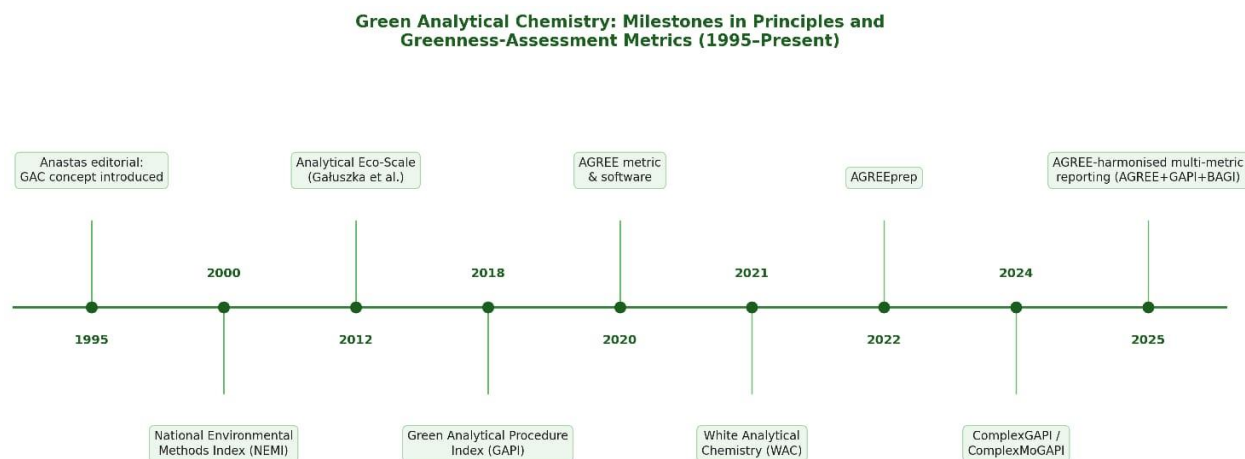


Figure 1: Evolution of Green Analytical Chemistry principles and greenness-assessment metrics from 1995 to the present.

2.2 The Twelve Principles of Green Analytical Chemistry (SIGNIFICANCE)

A landmark contribution to the field was the formal codification of twelve principles of GAC, often remembered through the mnemonic SIGNIFICANCE, which mirror — but are not identical to — the twelve principles of green chemistry for synthesis. These principles collectively define what constitutes an environmentally responsible analytical procedure, spanning sample handling, reagent selection, automation, energy use, and waste minimisation.^[5,9,10] Table 1 summarises these principles with their specific relevance to pharmaceutical QC laboratories.

#	Principle	Relevance to Pharmaceutical QC
1	Direct analytical techniques should be applied to avoid sample treatment	Favors direct NIR/Raman tablet analysis over wet-chemical assay where scientifically justified
2	Minimal sample size and minimal number of samples	Supports micro-sampling in stability and content-uniformity testing
3	In-situ measurements should be performed	Encourages PAT-based in-line monitoring instead of off-line lab testing
4	Integration of analytical processes and operations saves energy and reduces reagent use	Supports hyphenated and automated QC platforms (e.g., online SPE-LC)
5	Automated and miniaturized methods should be selected	Drives adoption of UHPLC, microfluidic chips, and miniaturized extraction
6	Derivatization should be avoided	Favors native-fluorescence/UV detection over derivatization-based assays
7	Generation of a large volume of analytical waste should be avoided and proper management of analytical waste should be provided	Central to solvent-reduction strategies in routine HPLC/UHPLC QC testing
8	Multi-analyte or multi-parameter methods are preferred over methods using one analyte at a time	Supports stability-indicating multi-drug assays in combination products
9	The use of energy should be minimized	Relevant to column temperature, instrument idle-time, and lyophilization steps
10	Reagents obtained from renewable sources should be preferred	Encourages bio-based solvents (ethanol, ethyl lactate) in mobile phases
11	Toxic reagents should be eliminated or replaced	Central driver for replacing acetonitrile/chlorinated solvents
12	The safety of the operator should be increased	Reduces occupational exposure risk in routine QC laboratories

It is important to emphasise that these principles are not absolute mandates but a decision-making framework. A pharmaceutical analyst applying GAC must weigh greenness improvements against method robustness, regulatory acceptability, and fitness for purpose — an approach later formalised as White Analytical Chemistry (WAC), which explicitly reconciles green (ecological), red (analytical performance), and blue (practicality/economic) criteria into a single holistic assessment.^[28]

3. Metrics and Tools for Assessing Analytical Greenness

Because greenness is a multidimensional property, a wide array of quantitative and semi-quantitative metrics has been developed to enable objective, reproducible comparison of analytical procedures. These tools differ in their scope (whole procedure vs. sample-preparation-only), their output format (numerical score, colour-coded pictogram, or radial chart), and the depth of detail they provide about specific weaknesses in a method. This section reviews the four tools most commonly applied in pharmaceutical QC literature.

3.1 National Environmental Methods Index (NEMI) and Analytical Eco-Scale

The Analytical Eco-Scale, proposed by Gałuszka, Migaszewski, Konieczka, and Namieśnik, was among the first semi-quantitative greenness tools designed specifically for analytical (as opposed to synthetic) procedures. It assigns penalty points for deviations from an ideal green analysis — accounting for reagent type and amount, occupational hazard, energy consumption, and waste generation — and subtracts the cumulative penalty from a maximum score of 100. Methods scoring above 75 are considered 'excellent green analysis', 50–75 'acceptable', and below 50 'inadequate'. Its simplicity has made it one of the most widely applied tools in pharmaceutical method validation reports, although it does not visually communicate which specific step of the analysis is the weakest link.^[5,6]

3.2 Green Analytical Procedure Index (GAPI)

GAPI, introduced by Płotka-Wasyłka in 2018, evaluates the green character of an entire analytical methodology — from sample collection and preservation through to final determination — using a pictogram composed of five linked pentagons, each subdivided into segments coloured green (low impact), yellow (moderate impact), or red (high impact). Unlike the single-number Eco-Scale, GAPI's visual format allows an analyst to identify, at a glance, exactly which stage of the procedure (e.g., sample preparation vs. instrumental analysis) contributes most to the environmental burden, making it particularly valuable for method redesign and continuous improvement initiatives in QC laboratories.^[9]

3.3 Analytical GREENness (AGREE) Metric and Software

AGREE, developed by Pena-Pereira, Wojnowski, and Tobiszewski in 2020, represents a significant advance by directly operationalising all twelve SIGNIFICANCE principles into a single, weighted, 0–1 scale. Using free open-source software, each of the twelve criteria is scored and displayed as a coloured segment of a circular pictogram, with the overall score shown at the centre — green tones near 1.0 indicating high greenness and red tones near 0 indicating poor greenness. Because analysts can assign custom weights to each principle according to laboratory-specific priorities, AGREE offers considerable flexibility while retaining a single, easily comparable output value, and it has rapidly become the most cited greenness metric in recent pharmaceutical analytical literature.^[8,11,12]

3.4 Complementary and Emerging Tools

Several complementary tools extend or refine the core metrics above. AGREEprep applies an analogous ten-criteria framework specifically to the sample-preparation stage, addressing a gap left by AGREE, which primarily emphasises the instrumental determination step. ComplexGAPI and ComplexMoGAPI extend the GAPI pictogram to more complex, multi-stage or hyphenated procedures with an integrated numerical scoring system. Blue Applicability Grade Index (BAGI) evaluates the practicality dimension (cost, instrument availability, throughput), while White Analytical Chemistry (WAC) — via its RGB12 algorithm — merges green (ecological), red (analytical performance), and blue (practicality) criteria into a unified 'whiteness' assessment, explicitly acknowledging that a method cannot be judged sustainable unless it is simultaneously accurate, robust, and economically viable.^[12,28] Table 2 compares the principal features of these tools.

Tool	Year / Author	Output Format	Key Strength	Key Limitation
Eco-Scale	2012, Gałuszka et al.	Single numeric score (0–100)	Simple, widely adopted	No stage-wise breakdown
GAPI	2018, Płotka-Wasyłka	5-pentagon colour pictogram	Whole-procedure visual mapping	Semi-quantitative only
AGREE	2020, Pena-Pereira et al.	Circular 0–1 pictogram + score	Directly maps 12 GAC principles; software-based	Weighting is subjective
AGREEprep	2022, Wojnowski et al.	Circular pictogram (10 criteria)	Dedicated to sample preparation	Needs pairing with AGREE for full method
ComplexGAPI / ComplexMoGAPI	2021 / 2024	Extended pictogram + score	Handles hyphenated/multi-step methods	More complex scoring
White Analytical Chemistry (RGB12)	2021, Nowak et al.	Red-Green-Blue composite score	Balances greenness with performance & practicality	Newer, less standardized

A recurring theme across recent literature — including a 2025 tutorial perspective on current and future metric tools — is that no single metric is universally sufficient; best practice in pharmaceutical method validation increasingly involves reporting two or more complementary scores (e.g., AGREE plus GAPI, or AGREE plus BAGI) to give a fuller picture of both environmental and practical performance.^[26]

4. Green Sample Preparation Strategies

Sample preparation is frequently the single most reagent- and labour-intensive stage of a pharmaceutical QC assay, and is therefore a priority target for greening. The 'Ten Principles of Green Sample Preparation' emphasise in-situ and direct treatment, minimal sample and reagent amounts, safer solvents, integration and automation of the sample-preparation and separation steps, and selection of bio-based, biodegradable, or reusable materials.^[21]

4.1 Miniaturized and Microextraction Techniques

Solid-phase microextraction (SPME), dispersive liquid–liquid microextraction (DLLME), and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) methodologies have all been adapted from environmental and food analysis into pharmaceutical impurity profiling and bioanalytical QC, reducing organic solvent consumption from tens of millilitres to microlitre volumes while often improving selectivity through more efficient analyte pre-concentration. Comparative recovery studies have demonstrated that SPME can achieve recovery rates exceeding 90% with markedly shorter

extraction times and lower environmental impact than classical liquid–liquid extraction, making it an attractive alternative for trace-level impurity and degradation-product analysis.^[21]

4.2 Deep Eutectic Solvents and Natural Deep Eutectic Solvents (NADES)

Deep eutectic solvents (DES), and particularly natural deep eutectic solvents (NADES) formed from primary metabolites such as sugars, organic acids, and amino acids, have emerged as compelling green alternatives to conventional organic extraction solvents. NADES are characterised by negligible vapour pressure, low toxicity, high biodegradability, tunable polarity, and — notably for pharmaceutical applications — the capacity to be biosynthesised and metabolised by living organisms, conferring excellent biocompatibility. Studies applying NADES to the extraction of multi-bioactive components from herbal pharmaceutical preparations have demonstrated extraction efficiencies equal to or exceeding those of conventional solvents such as methanol or ethanol, while eliminating the associated hazard and disposal burden¹⁸. Beyond extraction, NADES are increasingly explored as green reaction media, solubility enhancers in formulation, and even as active pharmaceutical ingredient (API) delivery vehicles, reflecting a convergence between green sample preparation and green pharmaceutical technology more broadly.

4.3 Solvent-Free and Direct Analysis Approaches

Where scientifically justified, direct analysis without sample pre-treatment represents the 'greenest' possible approach, fully satisfying SIGNIFICANCE principle 1. This is exemplified by direct powder or tablet analysis using near-infrared (NIR) or Raman spectroscopy, and by ambient ionisation mass spectrometry techniques (Section 6.3) that permit analysis of intact tablets, ointments, or raw powders without extraction, dissolution, or derivatisation. The elimination of a sample-preparation step not only reduces solvent and reagent consumption to zero but also shortens total analysis time — an important consideration for real-time release testing within a PAT framework.^[19,20]

5. Green Chromatographic and Electrophoretic Techniques in Pharmaceutical QC

5.1 Green HPLC: Solvent Substitution and Micellar Approaches

Reversed-phase HPLC remains the workhorse technique of pharmaceutical QC, and consequently the primary target of green method redesign. Strategies include substituting acetonitrile with ethanol, isopropanol, or ethyl lactate; employing micellar liquid chromatography with aqueous surfactant mobile phases that eliminate organic solvent almost entirely; reducing column internal diameter and particle size to shorten run time and lower total solvent consumption; and lowering flow rates without loss of resolution. Numerous validated case studies — including green stability-indicating and quality-by-design (QbD)-optimised RP-HPLC methods for drugs such as metoclopramide, cinnarizine/domperidone, and isosorbide dinitrate/hydralazine — have demonstrated that such substitutions can achieve Eco-Scale scores above 75 and AGREE scores above 0.7 while fully satisfying ICH validation criteria for linearity, accuracy, precision, and robustness.^[2,3,4,7,26]

5.2 Ultra-High-Performance Liquid Chromatography (UHPLC)

UHPLC, employing sub-2-micron stationary-phase particles under higher operating pressures, achieves markedly shorter run times and lower mobile-phase consumption per injection compared with conventional HPLC, translating directly into reduced solvent waste and energy use per sample analysed. Comparative greenness assessments consistently rank UHPLC methods as substantially 'greener' than their HPLC counterparts on Eco-Scale, GAPI, and AGREE metrics simultaneously, while typically delivering equal or superior chromatographic resolution — an unusual

case in which greenness and analytical performance improve in tandem rather than trading off against one another.^[1,6,22]

5.3 Supercritical Fluid Chromatography (SFC)

Supercritical fluid chromatography, using supercritical carbon dioxide as the primary mobile-phase component (often modified with a small proportion of polar co-solvent such as methanol), is one of the most intrinsically green separation techniques available to pharmaceutical analysts. Because CO₂ is non-toxic, non-flammable, inexpensive, and readily recovered/recycled after depressurisation, SFC can reduce organic solvent consumption by up to 90% and energy usage by roughly half relative to conventional HPLC, while offering faster analyses, orthogonal selectivity to reversed-phase LC, and, critically, outstanding chiral resolving power for enantiomeric impurity profiling. Modern ultra-high-performance SFC (UHPSFC) instrumentation now rivals UHPLC in throughput and resolution, and SFC-MS hyphenation has extended its applicability to bioanalysis, impurity identification, and complex formulation matrices.^[13,14,15,32,33]

5.4 Capillary Electrophoresis (CE)

Capillary electrophoresis achieves separation using microlitre-to-nanolitre sample and buffer volumes within narrow-bore fused-silica capillaries, resulting in dramatically lower reagent and waste generation than conventional LC. A comprehensive 2019–2023 literature review of green CE and LC methods for pharmaceutical and biomedical analysis found that CE methods consistently score favourably across AGREE, GAPI, and BAGI metrics, with particular advantages in high-resolution, high-efficiency separations of ionic and chiral pharmaceutical compounds, peptides, and biopharmaceuticals — an increasingly important application area as biologics constitute a growing share of the global pharmaceutical pipeline.^[11]

5.5 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC offers an inherently parallel, low-solvent-consumption platform in which multiple samples and standards are developed simultaneously on a single plate, sharply reducing per-sample mobile-phase use compared with column-based LC. Recent green HPTLC methodologies incorporating environmentally benign developing solvents and micellar systems have been successfully validated for stability-indicating assays of drugs such as safinamide, demonstrating that HPTLC densitometry can match ion-pair HPLC-DAD performance while substantially lowering the environmental footprint — an attractive proposition for routine identity and purity screening in resource-limited QC settings.^[25]

5.6 Comparative Overview of Green Chromatographic and Electrophoretic Techniques

Sections 5.1–5.5 examined each separation technique individually; Tables 3 and 4 consolidate these findings into a single, publication-ready comparative framework spanning advantages/disadvantages, solvent consumption, cost, greenness scores, and industrial applicability, followed by a criterion-by-criterion comparison across sensitivity and throughput. Together, they are intended to support method-selection decisions during green method redevelopment or new-method design in pharmaceutical QC laboratories.

Table 3: Comparative advantages, disadvantages, solvent consumption, cost, greenness, and industrial applicability of green HPLC, UHPLC, SFC, CE, and HPTLC.

Parameter	Conventional Green HPLC /	UHPLC	SFC	CE	HPTLC (reference)
Key advantages	Universal, well-validated; green solvent substitution (ethanol, ethyl lactate) cuts hazard without new hardware	Sub-2- μ m particles give faster runs, sharper peaks, lower per-injection solvent use than HPLC	scCO ₂ mobile phase is non-toxic, cheap, recyclable; up to ~90% less organic solvent; strong chiral resolving power	Nanolitre-to-microlitre volumes; minimal reagent/waste; excellent for ionic, chiral and biopharmaceutical analytes	Parallel multi-sample development on one plate; very low mobile-phase volume per sample
Key disadvantages	Highest solvent/energy footprint of the group if not greened; ACN/MeOH-dependent legacy methods still dominate	Requires dedicated UHPLC hardware (high back-pressure); method transfer from HPLC needs re-validation	High capital cost instrumentation; co-solvent optimisation can be complex; less familiar to routine QC staff	Lower loading capacity/sensitivity for some analytes; migration-time reproducibility needs careful control	Lower sensitivity/resolution than column LC for complex mixtures; semi-quantitative unless densitometry used
Typical solvent consumption per run	10–30 mL (conventional); 3–10 mL (greened)	1–5 mL	<2 mL organic co-solvent (bulk phase is recycled CO ₂)	<0.01–0.1 mL (buffer only)	~2–5 mL shared across an entire plate of samples
Relative instrument / running cost	Low–moderate capital; moderate running cost (solvent + waste disposal)	Moderate–high capital; lower running cost per sample (speed, solvent)	High capital (specialised pumps/back-pressure regulator); low running cost (CO ₂ is cheap)	Low–moderate capital; very low running cost (minimal reagents)	Low capital; very low running cost per sample
Illustrative greenness score	Eco-Scale ~58 (conventional) / ~74 (greened); AGREE ~0.48–0.66	Eco-Scale ~82; AGREE ~0.74	Eco-Scale ~88; AGREE ~0.82 (typically the greenest LC-type technique)	Eco-Scale ~85; AGREE ~0.78	Eco-Scale ~78; AGREE ~0.70
Industrial applicability in pharmaceutical QC	Universal; compendial (USP/Ph. Eur.) methods overwhelmingly HPLC-based; easiest regulatory acceptance	Growing compendial acceptance; well-suited to high-throughput batch release and stability testing	Established for chiral/enantiomeric impurity profiling; expanding into routine assay via UHPSFC	Niche but growing for biologics, peptides and charged small molecules; limited compendial precedent	Widely used for rapid identity/purity screening, especially in resource-limited QC settings

Table 4: Criterion-by-criterion comparison of greenness score, sensitivity, throughput, cost, and representative pharmaceutical QC applications across the five techniques.

Criterion	HPLC	UHPLC	SFC	CE	HPTLC
Greenness (AGREE, 0–1)	0.48–0.66	0.70–0.76	0.78–0.85	0.74–0.80	0.66–0.72
Relative sensitivity	High (ng– μ g range with UV/MS)	High–very high (sharper peaks, better S/N)	High, especially with MS hyphenation	Moderate (short optical path length limits UV sensitivity)	Moderate (densitometry improves quantitation)
Relative throughput	Moderate (10–30 min/sample typical)	High (2–10 min/sample)	High (fast equilibration, short run times)	Moderate–high (short capillaries, fast fields)	Very high (multiple samples)

					developed in parallel)
Relative cost (capital + running)	Low capital / moderate running	Moderate–high capital / low running	High capital / low running	Low–moderate capital / very low running	Low capital / very low running
Representative pharmaceutical QC applications	Assay, related-substances, dissolution, stability-indicating methods (compendial workhorse)	High-throughput batch release, stability programmes, multi-analyte combination products	Chiral/enantiomeric impurity profiling, achiral impurity screening, SFC-MS bioanalysis	Ionic/charged APIs, peptides, biopharmaceuticals, chiral separations	Rapid identity and purity screening, stability-indicating assays, resource-limited settings

As Table 3 and Table 4 illustrate, no single technique is universally superior: SFC and CE offer the strongest intrinsic greenness profiles but carry higher capital cost or narrower compendial precedent, while HPLC remains the most broadly validated and regulator-familiar platform. UHPLC represents the most balanced compromise currently available for routine batch-release testing, combining substantial greenness gains with minimal disruption to existing regulatory and operational infrastructure, whereas HPTLC offers a particularly favourable cost-to-greenness ratio for high-volume identity and purity screening.

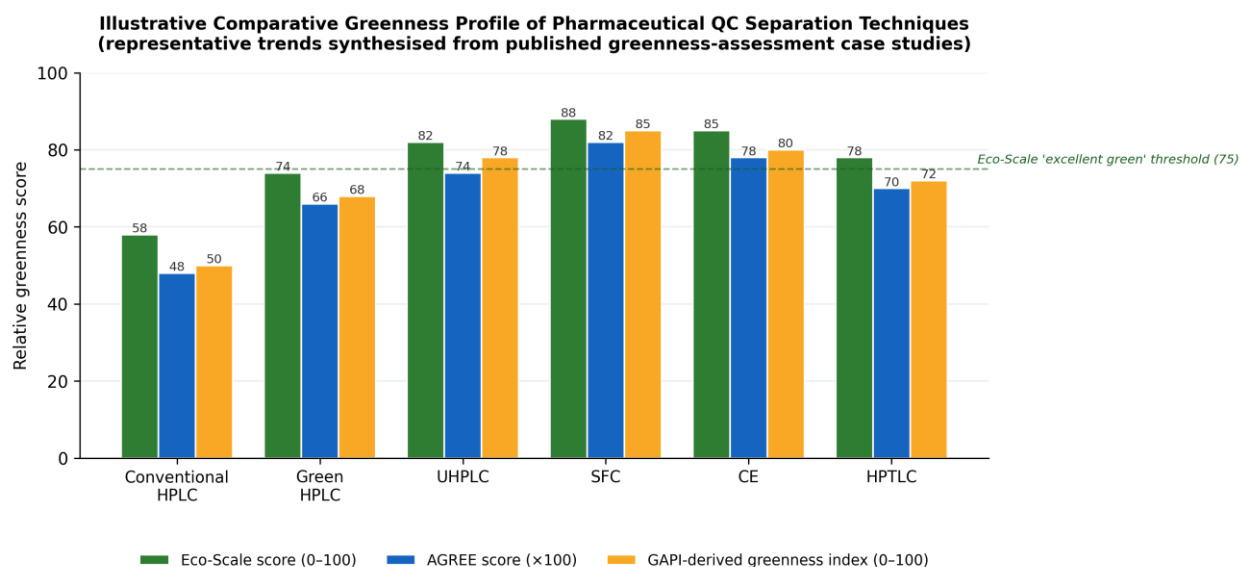


Figure 2: Illustrative comparative greenness profile of common pharmaceutical QC analytical techniques across the Eco-Scale, GAPI, and AGREE metrics (representative trends synthesised from published greenness-assessment case studies; values are illustrative rather than a single dataset).

6. Spectroscopic and Ambient Ionization Techniques

6.1 UV-Visible Spectrophotometry

UV-Vis spectrophotometry remains among the greenest instrumental techniques available, typically requiring only aqueous or minimal-organic-solvent sample dilution and no derivatisation for many chromophoric APIs. Recent smart chemometric spectrophotometric approaches — combining orthogonal partial least squares (OPLS), genetic-algorithm variable selection (GA-OPLS), and interval selection (i-OPLS) — have extended the applicability of UV-Vis to simultaneous multi-component assays of drug combinations and their impurities, such as proton-pump inhibitor

combination products, achieving selectivity historically associated only with chromatographic separation, while retaining the minimal-reagent footprint characteristic of spectrophotometric analysis.^[23]

6.2 Near-Infrared (NIR) and Raman Spectroscopy

NIR and Raman spectroscopy are non-destructive, reagent-free vibrational techniques capable of direct analysis of intact tablets, capsules, and powder blends, eliminating sample preparation entirely. NIR spectroscopy, validated extensively for pharmaceutical identity testing, content uniformity, and moisture determination, exploits overtone and combination bands of molecular vibrations and is particularly well-suited to rapid, high-throughput tablet screening and counterfeit/falsification detection. Raman spectroscopy offers complementary, generally sharper spectral features and superior spatial resolution for chemical imaging applications, making it valuable for mapping API distribution within tablets and for real-time in-line process monitoring. Both techniques require multivariate chemometric calibration (partial least squares regression, principal component analysis) to convert raw spectra into quantitative or qualitative results, linking spectroscopic greenness directly to the chemometrics discussed in Section 8.^[19,20,34,35]

6.3 Ambient Ionization Mass Spectrometry

Ambient ionisation techniques — including desorption electrospray ionisation (DESI), direct analysis in real time (DART), and related desorption/ionisation methods — permit direct mass-spectrometric analysis of solid pharmaceutical materials (tablets, ointments, raw powders) under ambient conditions without chromatographic separation, extraction, or derivatisation. Beyond dramatically reducing solvent consumption and analysis time, these techniques enable high-throughput reaction monitoring during process development and rapid screening for counterfeit or substandard medicines directly from dosage-form surfaces — an emerging application with significant implications for pharmacovigilance and supply-chain quality assurance in resource-constrained settings.

7. Miniaturization, Microfluidics, and Lab-on-Chip Systems

Microfluidic 'lab-on-a-chip' platforms integrate sample handling, reaction, separation, and detection within a single micro-fabricated device, typically consuming nanolitre-to-microlitre volumes of sample and reagent — several orders of magnitude less than conventional benchtop instrumentation. In pharmaceutical analysis, microfluidic chip technology (termed 'Pharm-Lab-on-a-Chip' in recent literature) has been applied to drug purity and identity screening, dissolution-related mechanistic studies, high-throughput reaction optimisation during drug synthesis, and even micro-scale enantiomeric separation, offering the combined benefits of miniaturisation, parallelisation, and rapid analysis time.^[29,30,31]

Despite compelling proof-of-concept demonstrations spanning more than two decades, widespread routine adoption of microfluidic platforms in regulated pharmaceutical QC laboratories has remained limited, constrained by challenges in device reproducibility, regulatory validation of non-standard hardware, and integration with existing laboratory information management systems (LIMS). Nonetheless, as 3D-printing and open-hardware fabrication methods mature and lower the barrier to in-house device development, microfluidic and lab-on-chip approaches represent one of the most promising frontiers for further reducing the environmental footprint of pharmaceutical analysis while simultaneously enabling point-of-care and decentralised quality testing.^[29,30]

8. Integration of Green Analytical Chemistry with PAT, QbD, Chemometrics, and Artificial Intelligence

8.1 Process Analytical Technology (PAT)

PAT, formalised by the US FDA in 2004 as 'a system for designing, analyzing, and controlling manufacturing through timely measurements ... of critical quality and performance attributes,' represents a philosophical shift from end-product (off-line) testing toward continuous, real-time quality assurance built into the manufacturing process itself. NIR and Raman spectroscopy are the dominant PAT sensor technologies, enabling in-line or at-line monitoring of blend uniformity, moisture content, granulation endpoint, and tablet coating thickness, thereby reducing the number of discrete off-line QC samples — and their associated solvent and reagent consumption — that must be tested. The European Medicines Agency's PAT initiative and the ICH Q8–Q11 guideline series have reinforced this shift globally, embedding PAT within a Quality by Design framework across the US, EU, and Japan.^[16,17]

8.2 Quality by Design (QbD) and Analytical Quality by Design (AQbD)

QbD asks manufacturers and analysts to define quality objectives — including, increasingly, sustainability objectives — upfront, and to build processes and methods scientifically capable of meeting them, rather than discovering deficiencies after the fact through end-testing. Applied to analytical method development, this becomes Analytical Quality by Design (AQbD): an Analytical Target Profile (ATP) is defined that explicitly incorporates greenness criteria alongside conventional accuracy/precision requirements, Design of Experiments (DoE) is used to systematically optimise method parameters (mobile-phase composition, flow rate, column temperature) within a defined design space, and the resulting Method Operable Design Region (MODR) guarantees robust performance across the sustainable operating window. Multiple pharmaceutical case studies employing principal component analysis (PCA) and DoE-driven green AQbD approaches have demonstrated that solvent consumption can be reduced substantially while simultaneously improving robustness, illustrating that green and QbD philosophies are highly complementary rather than competing priorities.^[3,4,7]

8.3 Chemometrics, Machine Learning, and Artificial Intelligence

Chemometric multivariate calibration (PLS, PCA, PARAFAC) has long been indispensable for converting complex spectroscopic signals (NIR, Raman, UV-Vis) into quantitative results without wet-chemical reference methods, directly enabling several of the green spectroscopic techniques discussed in Section 6. More recently, machine learning and deep learning approaches — random forest, support vector machines, and artificial neural networks — have been layered atop classical chemometrics to improve predictive accuracy for complex, non-linear spectral-property relationships, automate method-development decision-making, and even predict the likely greenness score of a candidate method before it is experimentally executed, accelerating the AQbD design cycle.^[27,36] AI-assisted in-silico toxicity prediction tools are additionally being coupled with green micellar chromatographic methods to simultaneously assess impurity safety and method sustainability within a single integrated workflow, illustrating the convergence of computational and green-analytical thinking in contemporary pharmaceutical QC.

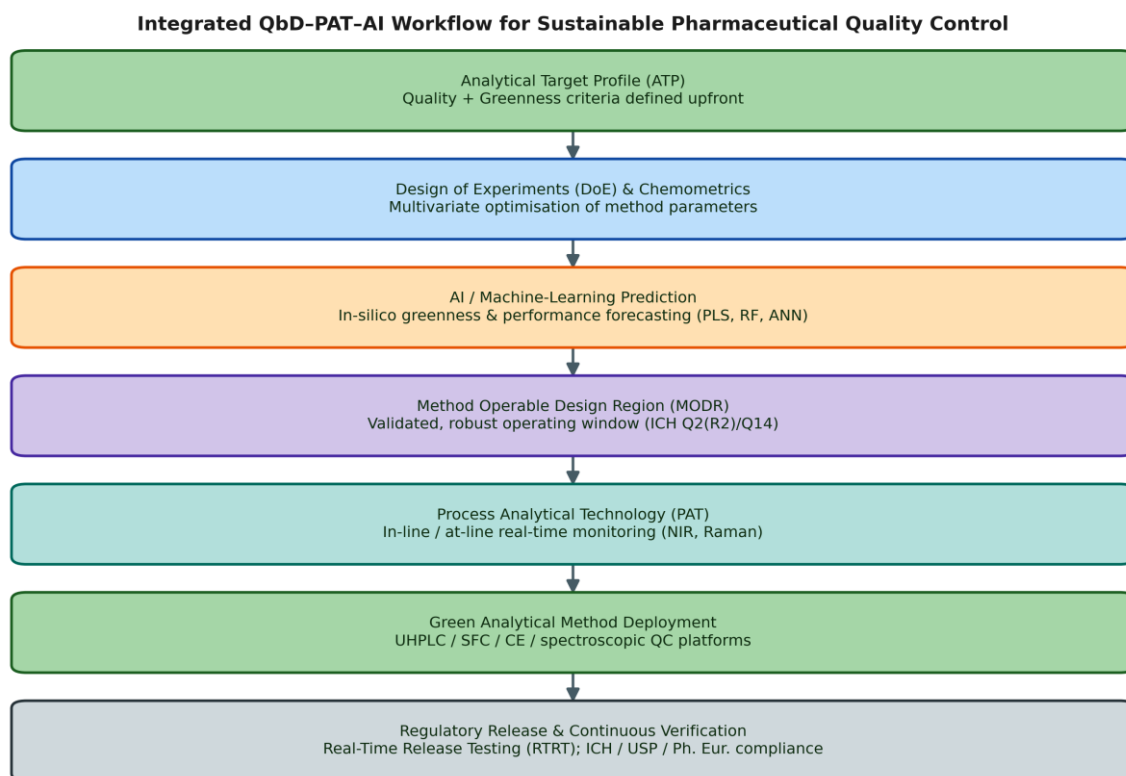


Figure 3: Integrated workflow illustrating how Green Analytical Chemistry principles are embedded within a QbD-PAT-AI driven pharmaceutical quality-control system, from Analytical Target Profile definition through regulatory release.

9. Industrial Implementation: Opportunities and Challenges

9.1 Method Validation and Lifecycle Management

Every green method substitution — whether a solvent change, miniaturised extraction, or spectroscopic replacement of a wet-chemical assay — must be fully revalidated according to ICH Q2(R2)/Q14 criteria for specificity, linearity, accuracy, precision, range, detection/quantitation limits, and robustness before it can replace an existing pharmacopoeial or in-house QC method. Because regulatory filings typically reference a specific validated method, greening an established, approved method also triggers lifecycle-management considerations: post-approval change control, potential regulatory notification or approval requirements in each market where the product is registered, and bridging studies to demonstrate equivalence between the legacy and green methods. This regulatory burden is frequently cited as the single largest practical barrier to green method adoption in already-marketed products, even when the green alternative is scientifically superior in performance.

9.2 Technology Transfer

Transferring a greener method between sites — for example, from a research and development laboratory to a commercial QC laboratory, or between manufacturing sites in different countries — introduces additional variables: differences in instrument vendor/model (particularly relevant for UHPLC, SFC, and spectroscopic PAT sensors), local solvent/reagent availability and quality, and analyst training. Successful technology transfer of green methods requires robust, well-characterised design spaces (as provided by AQbD/MODR approaches) that explicitly define the acceptable operating ranges within which the method's greenness and performance are maintained across sites.

9.3 Economic Feasibility

While green methods frequently reduce solvent purchase and hazardous-waste disposal costs — sometimes substantially, given the rising cost of high-purity acetonitrile and increasingly stringent hazardous-waste disposal regulations — the initial capital investment in new instrumentation (SFC systems, UHPLC, PAT spectroscopic sensors, microfluidic platforms) can be significant. A full economic case for green method adoption must therefore account for total cost of ownership across the method lifecycle, including reduced analyst time (from faster UHPLC/SFC runs), lower waste-disposal fees, reduced regulatory risk from improved sustainability compliance, and potential savings from reduced batch-release cycle times when PAT-based real-time release testing replaces lengthy off-line QC testing sequences.

9.4 Workforce Training and Organisational Culture

The successful institutionalisation of GAC within a QC organisation depends heavily on analyst familiarity with greenness-assessment tools (Eco-Scale, GAPI, AGREE), chemometric software, and the underlying rationale for method changes. Academic pharmacy curricula, including M.Pharm - level pharmaceutical analysis coursework, increasingly incorporate GAC principles and greenness-metric calculation as core competencies, reflecting industry demand for analysts capable of both developing and critically evaluating sustainable analytical methods from the earliest stages of their careers.

10. Regulatory Landscape and Harmonization

10.1 International Council for Harmonisation (ICH)

The ICH Q8 (Pharmaceutical Development), Q9 (Quality Risk Management), Q10 (Pharmaceutical Quality System), and Q11 (Development and Manufacture of Drug Substances) guidelines collectively established the QbD and PAT framework that underpins much of the industrial GAC adoption discussed in Section 8, though none of these guidelines explicitly mandate or even formally reference environmental sustainability as a quality attribute. More recently, ICH Q2(R2) and the new Q14 guideline on Analytical Procedure Development explicitly endorse AQbD principles and provide a harmonised framework for defining Analytical Target Profiles and Method Operable Design Regions — creating, for the first time, a natural regulatory 'hook' through which sustainability criteria could be formally incorporated into method-development documentation, even though explicit greenness requirements remain absent from the guideline text itself.

10.2 United States Pharmacopeia (USP) and European Pharmacopoeia (Ph. Eur.)

Neither the USP General Chapters nor the European Pharmacopoeia currently mandate the use of green solvents or the reporting of greenness metrics for compendial methods, and the vast majority of official pharmacopoeial assay and impurity methods still specify acetonitrile-based mobile phases inherited from decades-old method development. However, both pharmacopoeias permit the use of alternative, validated methods provided equivalence to the compendial method is demonstrated, creating a regulatory pathway — but not yet an obligation — for greener alternatives in routine compendial testing. Growing engagement from pharmacopoeial expert committees with green chemistry principles, and periodic revision cycles that increasingly reference more contemporary green solvent options, suggest gradual movement toward more formal sustainability integration in future compendial revisions.

10.3 Regional and Environmental Regulatory Drivers

Beyond pharmacopoeial and ICH frameworks, broader environmental regulations — including REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in the European Union and analogous hazardous-substance and waste-disposal regulations elsewhere — indirectly but powerfully incentivise green method adoption by increasing the cost and administrative burden of using and disposing of hazardous solvents such as acetonitrile, dichloromethane, and chloroform. Corporate Environmental, Social, and Governance (ESG) commitments made by major pharmaceutical manufacturers have likewise become an increasingly significant driver, with several companies publicly committing to solvent-reduction and green-method targets independent of any formal regulatory mandate.

10.4 Gaps in Standardization

A persistent and widely acknowledged gap is the absence of a single, internationally harmonised, regulator-endorsed greenness metric or reporting standard. The coexistence of multiple competing tools (Eco-Scale, GAPI, AGREE, WAC, and their many derivatives, see Table 2) — each with different scoring philosophies and weighting schemes — makes cross-study and cross-laboratory comparison difficult, and no regulatory agency currently requires or formally recognises any specific greenness score as part of a marketing-authorisation or compendial-method submission. Addressing this gap through coordinated action between the ICH Analytical Procedures Discussion Group, pharmacopoeial expert committees, and the broader green analytical chemistry research community represents one of the most important unmet needs identified throughout this review.

11. Challenges and Future Research Directions

- Harmonisation of greenness metrics: development of a single, regulator-recognised, internationally harmonised greenness-reporting standard, potentially issued jointly by ICH and major pharmacopoeial authorities.
- Extension of AQbD frameworks to formally embed sustainability criteria within the Analytical Target Profile as a mandatory, rather than optional, quality attribute.
- Further validation and regulatory acceptance of PAT-based real-time release testing (RTRT) as a full or partial replacement for traditional off-line QC testing across a broader range of dosage forms and drug classes.
- Expanded application of artificial intelligence and machine learning for automated, in-silico prediction of both analytical performance and greenness score during early method-development stages, reducing experimental trial-and-error.
- Continued development and pharmaceutical-scale validation of natural deep eutectic solvents (NADES) and other bio-based solvent systems as drop-in replacements for conventional chromatographic mobile phases.
- Greater industrial and regulatory validation of microfluidic and lab-on-chip platforms to move these technologies from academic proof-of-concept toward routine, GMP-compliant QC deployment.
- Investment in workforce training and curriculum reform, particularly at the M.Pharm and Ph.D. level, to ensure the next generation of pharmaceutical analysts is fluent in both green-chemistry principles and their practical, validated application in regulated environments.
- Life-cycle assessment (LCA) studies quantifying the true cradle-to-grave environmental impact of green analytical methods, extending beyond laboratory-level greenness metrics to encompass instrument manufacture, energy sourcing, and end-of-life disposal.

12. CONCLUSION

Green Analytical Chemistry has evolved from a niche academic concept into a mainstream, scientifically rigorous, and increasingly industrially adopted approach to pharmaceutical quality control. The convergence of standardised greenness metrics (Eco-Scale, GAPI, AGREE, and their derivatives), environmentally benign separation and spectroscopic technologies (green HPLC/UHPLC, SFC, CE, NIR, Raman, ambient MS), and modern quality frameworks (QbD, PAT, AQbD, chemometrics, and AI) has demonstrated repeatedly that sustainability and analytical excellence are not competing objectives but mutually reinforcing ones. Nevertheless, meaningful barriers remain — regulatory harmonisation gaps, revalidation and technology-transfer burdens, capital investment requirements, and the absence of a universally recognised greenness-reporting standard — that will require sustained, collaborative effort among academia, industry, and regulatory authorities to fully overcome. As global regulatory bodies, pharmacopoeial authorities, and pharmaceutical manufacturers increasingly align around shared sustainability objectives, Green Analytical Chemistry is poised to become not an optional enhancement but a foundational expectation of twenty-first-century pharmaceutical quality assurance.

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