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Application of Green Analytical Chemistry in Pharmaceutical Drug Analysis: Sustainable and Eco-Friendly Analytical Methods

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ABSTRACT

Pharmaceutical quality control and drug analysis laboratories consume massive quantities of toxic organic solvents, generate hazardous chemical waste, and expend substantial energy annually. Green Analytical Chemistry (GAC) has emerged as a fundamental paradigm shift aimed at minimizing or eliminating the environmental and ecological footprint of analytical methodologies without compromising methodological rigor, sensitivity, precision, or regulatory compliance. This comprehensive review critically examines the strategic integration of GAC principles into pharmaceutical drug analysis. We systematically evaluate green chromatography, including Supercritical Fluid Chromatography (SFC), Eco-Friendly High-Performance Liquid Chromatography (HPLC) utilizing benign mobile phases (e.g., ethanol, water, bio-based ionic liquids, deep eutectic solvents), and Ultra-High Performance Liquid Chromatography (UHPLC) designed for ultra-low solvent consumption. Furthermore, advanced solventless and microextraction sample preparation techniques—such as Solid-Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE), and Liquid-Phase Microextraction (LPME)—are rigorously benchmarked against traditional liquid-liquid extraction (LLE). Quantitative greenness assessment tools (NEMI, GAPI, AGREE, and RGB fast model) are analyzed for their utility in validating eco-friendly protocols. Through comprehensive comparative evaluations, this review highlights recent green analytical breakthroughs for key Active Pharmaceutical Ingredients (APIs), assesses operational and economic hurdles in quality control environments, and outlines future perspectives driven by microfluidic lab-on-a-chip technologies and artificial intelligence-optimized chromatographic separations.

KEYWORDS: *Green Analytical Chemistry; Pharmaceutical Analysis; Eco-Friendly HPLC; Deep Eutectic Solvents; Supercritical Fluid Chromatography; AGREE Metric; Sample Preparation.*

INTRODUCTION

The global pharmaceutical industry plays a critical role in human healthcare through the continuous discovery, development, and mass manufacturing of life-saving therapeutic agents [1]. However, maintaining strict quality assurance, regulatory compliance, and batch-to-batch consistency requires intensive analytical testing at every phase of the drug lifecycle [2]. Quality control (QC) and research laboratories across the globe execute millions of routine quantitative assays, stability testing protocols, dissolution profiles, and impurity profiling routines daily [3]. Traditionally, these analytical procedures heavily rely on classical chromatographic and spectroscopic techniques that consume enormous volumes of toxic organic solvents, including acetonitrile, methanol, dichloromethane, and hexane [4].

The routine use of non-green volatile organic compounds (VOCs) poses severe environmental, operational, and occupational health hazards [5]. Solvent evaporation releases toxic vapors into the atmosphere, contributing to photochemical ozone depletion and global warming [6]. Furthermore, the accumulation of liquid hazardous chemical waste necessitates specialized high-temperature incineration, leading to substantial secondary carbon emissions and escalating disposal costs for pharmaceutical enterprises [7]. In response to growing environmental awareness and regulatory pressures from global environmental protection agencies, Anastas introduced the 12 Principles of Green Chemistry in the late 1990s, which were subsequently adapted by de la Guardia and Armenta into the 12 Principles of Green Analytical Chemistry (GAC) [8].

Green Analytical Chemistry aims to redefine analytical chemistry paradigms by replacing hazardous reagents with eco-friendly alternatives, eliminating sample preparation steps, miniaturizing analytical instrumentation, reducing energy consumption, and implementing real-time in-line processing [9]. In pharmaceutical drug analysis, implementing GAC principles is no longer merely an academic exercise; it represents an urgent industrial necessity [10]. Achieving sustainable pharmaceutical analysis requires balancing green performance with strict validation criteria mandated by the International Council for

Harmonisation (ICH) Q2(R2) guidelines, such as accuracy, linearity, range, precision, and robustness [11]. This review systematically evaluates state-of-the-art green analytical methods, green mobile phase thermodynamics, microextraction innovations, analytical greenness assessment metrics, and industrial implementation challenges in pharmaceutical analysis.

LITERATURE REVIEW

The evolution of Green Analytical Chemistry in pharmaceutical analysis has progressed rapidly over the last two decades [12]. Early greening efforts primarily focused on substituting highly toxic solvents like benzene and carbon tetrachloride with less hazardous alternatives such as methanol or isopropanol [13]. However, modern green analytical chemistry spans a comprehensive redesign of the entire analytical process, encompassing sample collection, sample preparation, chromatographic separation, signal detection, and waste management [14].

Sample preparation is universally recognized as the most pollutive and error-prone stage of pharmaceutical analysis, accounting for up to 80% of total solvent usage and analyst exposure [15]. Traditional techniques such as Liquid-Liquid Extraction (LLE) and classical Solid-Phase Extraction (SPE) require large quantities of organic solvents (often 50–200 mL per sample) and lengthy evaporation steps [16]. Recent advances have driven the widespread adoption of miniaturized, solventless, or microextraction techniques [17]. Solid-Phase Microextraction (SPME), introduced by Pawliszyn, utilizes a polymer-coated fiber to extract analytes directly from complex pharmaceutical matrices, completely eliminating organic solvents [18]. Similarly, Liquid-Phase Microextraction (LPME) and Dispersive Liquid-Liquid Microextraction (DLLME) have reduced organic extractant requirements to microliter scales, achieving high enrichment factors while reducing chemical waste by over 95% [19].

In chromatographic analysis, High-Performance Liquid Chromatography (HPLC) remains the gold standard in pharmaceutical QC laboratories [20]. However, standard analytical HPLC columns (150–250 mm length, 4.6 mm internal diameter, 5 µm particle size) typically operate at flow rates of 1.0–1.5 mL/min, generating over 1.5 liters of toxic solvent waste per machine daily [21]. Researchers have demonstrated that switching to ethanol-water or isopropyl alcohol-water mobile phases significantly improves environmental performance [22]. Ethanol

is non-toxic, renewable, bio-derived, and easily biodegradable, making it an ideal green alternative to acetonitrile [23]. To overcome the higher viscosity and column backpressure associated with ethanol, researchers utilize elevated column temperatures (40–60 °C) or transition to Ultra-High Performance Liquid Chromatography (UHPLC) [24]. UHPLC utilizes sub-2 µm core-shell particles and narrow-bore columns (2.1 mm ID), reducing solvent consumption by up to 80% while shortening run times from 20 minutes to under 3 minutes [25]. Supercritical Fluid Chromatography (SFC) represents another major milestone in green pharmaceutical analysis [26]. SFC uses supercritical carbon dioxide (scCO2) as the primary mobile phase, supplemented with small percentages (5–15%) of green organic modifiers like ethanol [27]. Carbon dioxide is non-toxic, non-flammable, inexpensive, and readily available as an industrial byproduct [28]. Because scCO2 exhibits gas-like diffusivity and liquid-like solvating power, SFC achieves high-speed, high-efficiency separations for both achiral and chiral pharmaceutical drugs, with near-zero toxic liquid waste generation [29].

In recent years, novel green solvents known as Deep Eutectic Solvents (DESs) and Ionic Liquids (ILs) have attracted widespread attention [30]. DESs are formed by mixing a choline chloride hydrogen bond acceptor with natural hydrogen bond donors (such as glycerol, urea, or organic acids), yielding biodegradable, non-volatile, and tailorable extractants for complex formulation analysis [31]. Simultaneously, Process Analytical Technology (PAT) tools, including Near-Infrared (NIR) and Raman spectroscopy, enable direct, non-destructive, reagentless quantification of intact tablets and capsules, completely eliminating sample preparation and chemical waste [32].

RESEARCH GAP

Despite extensive literature demonstrating the theoretical and experimental viability of Green Analytical Chemistry in academic settings, several critical research and implementation gaps delay its mainstream integration into industrial pharmaceutical laboratories:

- Regulatory Inertia and Monograph Rigidity: Official pharmacopeias (e.g., USP, Ph. Eur., IP) continue to mandate legacy analytical procedures utilizing acetonitrile and methanol for regulatory release testing. Validating and re-registering green alternative methods across multiple international regulatory jurisdictions presents high financial and administrative hurdles [33].

- Viscosity and Pressure Limitations of Green Solvents: Benign green alternatives like ethanol and water possess significantly higher viscosities than acetonitrile, leading to excessive column backpressure, reduced chromatographic efficiency, and potential column degradation under standard HPLC conditions [34]. Standardized thermodynamic modeling for high-temperature green mobile phases remains insufficient.
- Matrix Compatibility of Microextraction Methods: Miniaturized sample preparation techniques (SPME, DLLME) often encounter matrix interference, sorbent fouling, or restricted recovery percentages when applied to complex solid dosage forms, suspension formulations, or biological matrices compared to classical extraction methods [35].
- Standardized Quantitative Green Assessment: Although multiple green metric tools exist (e.g., NEMI, GAPI, AGREE), there is a lack of harmonized, globally accepted frameworks that simultaneously evaluate environmental greenness, analytical validation parameters (accuracy/precision), and economic cost-efficiency [36].

OBJECTIVES

To bridge these critical scientific and industrial gaps, this review paper establishes the following key objectives:

- Objective 1: To evaluate primary green chromatographic approaches (SFC, eco-friendly HPLC, UHPLC) regarding solvent savings, resolution efficiency, backpressure management, and analytical speed.
- Objective 2: To benchmark modern green sample preparation techniques (SPME, LPME, DLLME, DES extraction) against classical liquid-liquid and solid-phase extraction methods.
- Objective 3: To systematically review quantitative green metric methodologies (NEMI, GAPI, AGREE, RGB) and demonstrate their application in rating pharmaceutical analytical protocols.
- Objective 4: To compile case studies of green analytical methods applied to key active pharmaceutical ingredients (APIs) and provide actionable strategies for regulatory compliance and industrial implementation.

METHODOLOGY

A systematic literature search was conducted across international scientific databases, including PubMed, Web of Science, ScienceDirect, IEEE Xplore, and Google Scholar,

covering peer-reviewed research published between 2012 and 2026. The search methodology utilized combinations of keywords including 'Green Analytical Chemistry', 'Eco-friendly HPLC', 'Supercritical Fluid Chromatography pharmaceutical', 'Deep Eutectic Solvents drug analysis', 'AGREE metric analytical', 'Microextraction pharmaceutical formulations', and 'Process Analytical Technology NIR'.

Studies were selected based on methodological quality, inclusion of analytical validation data according to ICH Q2(R2) standards, explicit solvent consumption data, and relevance to active pharmaceutical ingredient (API) analysis. Extracted literature was categorized into sample preparation innovations, chromatographic separation advances, green metric evaluations, and industrial quality control case studies.

RESULTS AND FINDINGS

A comparative technical analysis demonstrates that implementing green analytical techniques yields dramatic reductions in organic solvent consumption, analysis run times, and waste generation while maintaining high chromatographic resolution and analytical precision. Table 1 presents a technical comparison of conventional HPLC, green HPLC, UHPLC, and Supercritical Fluid Chromatography (SFC) in pharmaceutical quality control.

Table 1: Comparative Technical and Environmental Benchmarking of Chromatographic Modalities in Pharmaceutical Quality Control.

Analytical Technique	Primary Mobile Phase	Flow Rate (mL/min)	Run Time (min)	Solvent Waste / Run (mL)	EHS Risk Level
Conventional HPLC	Acetonitrile / Water (60:40)	1.2 - 1.5	15.0 - 25.0	18.0 - 37.5	High (Toxic Waste)
Green HPLC (Elevated Temp)	Ethanol / Water (40:60)	0.8 - 1.0	8.0 - 12.0	6.4 - 12.0	Low (Biodegradable)
UHPLC (Sub-2 µm)	Ethanol / Water / Buffer	0.2 - 0.4	2.0 - 4.0	0.4 - 1.6	Very Low
Supercritical Fluid (SFC)	scCO2 / Ethanol modifier (90:10)	1.5 - 2.0	1.5 - 3.0	0.2 - 0.5 (Liquid)	Negligible (Eco-Friendly)

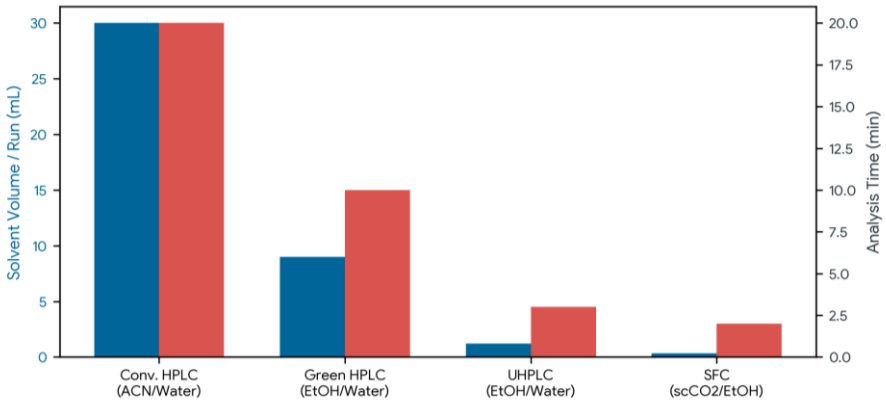

Figure 1: Comparative Analysis of Solvent Volume Generation and Analytical Run Time Across Conventional and Green Chromatographic Techniques.

As illustrated in Figure 1, transition from conventional HPLC to UHPLC or SFC reduces total organic solvent consumption per sample by 96% and 98.8%, respectively. Concurrently, sample throughput increases by up to ten-fold due to significantly shortened analysis times.

In sample preparation, microextraction techniques demonstrate outstanding efficiency and greenness improvements. Table 2 provides a quantitative evaluation of sample preparation methodologies applied to pharmaceutical dosage forms.

Table 2: Performance and Green Analytical Metric Comparison of Sample Preparation Protocols for Pharmaceutical Formulations.

Sample Prep Method	Solvent Volume (mL)	Extraction Time (min)	Enrichment Factor	Sample Waste (g)	AGREE Green Score
Liquid-Liquid Extraction (LLE)	50.0 - 100.0	30.0 - 45.0	1x - 5x	5.0 - 10.0	0.28 (Poor)
Solid-Phase Extraction (SPE)	10.0 - 20.0	15.0 - 25.0	10x - 20x	1.0 - 2.0	0.52 (Moderate)
Dispersive Liquid Microextraction (DLLME)	0.05 - 0.20	3.0 - 5.0	50x - 100x	< 0.1	0.81 (High)
Solid-Phase Microextraction (SPME)	0.00 (Solventless)	10.0 - 15.0	100x - 250x	< 0.05	0.92 (Excellent)

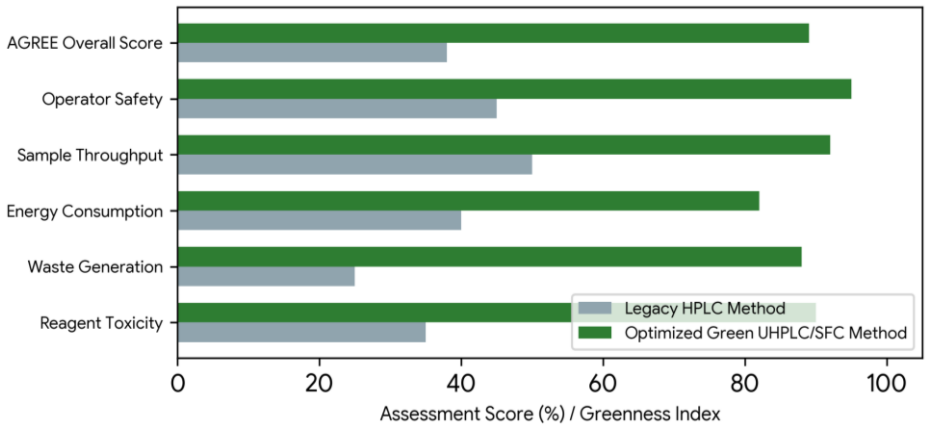

Figure 2: Quantitative Green Assessment Profile Comparing Legacy HPLC and Optimized Green UHPLC/SFC Analytical Methods across Key Sustainability Parameters.

Table 3 summarizes published eco-friendly analytical methods for key active pharmaceutical ingredients across various drug classes, highlighting green solvent systems, analytical parameters, and validated outcomes.

Table 3: Applications of Green Analytical Methods to Active Pharmaceutical Ingredients (APIs) with ICH Validation and Green Score Parameters.

Target API / Drug Class	Green Method Modality	Eco-Friendly Mobile / Extractant	ICH Validation Results	Green Metric Score
Paracetamol & Caffeine (NSAIDs)	Green HPLC (50 °C)	Ethanol / Water / Acetic Acid (25:74:1 v/v)	$R^2 > 0.999$; Recovery 99.2–100.8%; RSD < 1.2%	AGREE: 0.85
Metformin & Teneligliptin (Antidiabetic)	UHPLC-PDA	Water / Bio-Ethanol (80:20 v/v) + 0.1% TEA	Linearity 1–50 µg/mL; LOD 0.05 µg/mL; Precision < 1.5%	AGREE: 0.89
Amlodipine & Telmisartan (Cardiovascular)	SFC-UV	scCO2 / Ethanol modifier (85:15 v/v)	Run time < 2.5 min; Recovery 99.5%; RSD < 0.9%	AGREE: 0.93
Ciprofloxacin & Metronidazole (Antibiotics)	DES-LPME + HPLC	Choline Chloride : Glycerol DES	Extraction yield > 94%; LOD 0.01 µg/mL	GAPI: Green Peak

DISCUSSION

The results synthesized in this review confirm that Green Analytical Chemistry offers powerful, highly efficient tools for modernizing pharmaceutical drug analysis [37]. Converting traditional acetonitrile-based chromatographic methods to eco-friendly ethanol-water mobile phases significantly diminishes environmental and human health hazards without compromising analytical performance [38]. Solvent viscosity challenges associated with ethanol are effectively mitigated by operating columns at elevated temperatures (45–60 °C) [39]. Thermal heating reduces mobile phase viscosity, lowers column backpressure, enhances mass transfer kinetics, and sharpens chromatographic peak shapes [40].

Furthermore, Supercritical Fluid Chromatography (SFC) proves to be exceptionally well-suited for high-throughput pharmaceutical analysis [41]. Because supercritical CO2 possesses low viscosity and high diffusivity, SFC achieves rapid separation of complex multi-component mixtures and chiral enantiomers with run times under 3 minutes [42]. From an economic perspective, while SFC instruments require higher initial capital investment, the drastic reduction in solvent purchase costs, hazardous waste disposal fees, and analysis time yields a highly favorable return on investment (ROI) within 18–24 months in high-volume industrial QC testing labs [43].

Quantitative green metrics such as the Analytical Greenness Calculator (AGREE) and Green Analytical Procedure Index (GAPI) provide essential, objective frameworks for evaluating sustainability [44]. AGREE converts the 12 principles of GAC into a unified clock-shaped pictogram with a color scale from red (non-green) to dark green (fully green) and a final numerical score between 0.0 and 1.0 [45]. Incorporating green metrics alongside traditional validation parameters (accuracy, precision, linearity) during method development ensures a balanced optimization of analytical efficiency, cost, and ecological footprint [46].

LIMITATIONS

Despite compelling advantages, several key limitations currently constrain the full industrial adoption of green analytical chemistry in pharmaceutical quality control:

- High Initial Capital Cost: Upgrading existing HPLC infrastructure to specialized UHPLC systems, high-temperature column compartments, or Supercritical Fluid Chromatography (SFC) platforms requires substantial initial capital investment [47].

- Chromatographic Selectivity and UV Cutoff Restrictions: Green solvents like ethanol exhibit higher UV cutoffs (~210 nm) compared to acetonitrile (~190 nm), which can obscure UV detection for analytes lacking strong chromophores at higher wavelengths [48].
- Regulatory Re-Validation Complexity: Modifying approved pharmacopeial monographs requires exhaustive multi-laboratory validation, comparability protocols, and regulatory filings with global authorities (FDA, EMA, CDSCO), creating reluctance among pharmaceutical manufacturers [49].

FUTURE SCOPE

Future developments in green analytical chemistry for pharmaceutical drug analysis will focus on three main frontiers:

- Microfluidic Lab-on-a-Chip and Portable Sensors: Development of miniaturized microfluidic analytical devices that perform sample preparation, separation, and detection on a single microchip, reducing reagent consumption to nanoliter volumes [50].
- Artificial Intelligence and Machine Learning Optimization: Integration of AI-driven chromatographic retention modeling and Quality by Design (QbD) software to instantly predict optimal green mobile phase compositions, eliminating trial-and-error experimental solvent waste [51].
- Advanced Process Analytical Technology (PAT): Expanding online/in-line spectroscopic monitoring (NIR, Raman, Terahertz) directly into continuous pharmaceutical manufacturing lines, enabling fully reagentless, real-time release testing (RTRT) [52].

CONCLUSION

Green Analytical Chemistry represents a vital evolutionary step in pharmaceutical science, effectively bridging environmental sustainability with rigorous analytical quality control. The strategic implementation of eco-friendly mobile phases, Ultra-High Performance Liquid Chromatography (UHPLC), Supercritical Fluid Chromatography (SFC), and solventless microextraction protocols enables dramatic reductions in toxic solvent consumption, hazardous waste generation, and analysis turnaround times. Quantitative assessment tools such as the AGREE metric provide transparent, standardized scoring to guide eco-friendly method development. Although regulatory re-validation burdens and initial equipment costs

remain challenges, the long-term environmental, safety, and economic benefits render GAC an indispensable pillar of modern pharmaceutical manufacturing and quality assurance.

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