WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 12, 1822-1837. Review Article ISSN 2277–7105

GREEN ANALYTICAL CHEMISTRY IN RP-HPLC METHOD DEVELOPMENT FOR ANTIDIABETIC FIXED-DOSE COMBINATIONS: A COMPREHENSIVE REVIEW WITH SPECIAL REFERENCE TO METFORMIN HYDROCHLORIDE AND DAPAGLIFLOZIN

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Article Received on 15 May 2026,
Article Revised on 05 June 2026,
Article Published on 16 June 2026,

https://doi.org/10.5281/zenodo.20731172

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How to cite this Article: *Mr. Mohammad Zameeruddin, Ms. Pooja Bhimrao Lamdade. (2026). Green Analytical Chemistry In Rp-Hplc Method Development For Antidiabetic Fixed-Dose Combinations: A Comprehensive Review With Special Reference To Metformin Hydrochloride And Dapagliflozin. World Journal of Pharmaceutical Research, 15(12), 1822–1837.

ABSTRACT

The growing prevalence of type 2 diabetes mellitus (T2DM) and the increasing clinical preference for fixed-dose combinations (FDCs) have necessitated the development of reliable, sensitive, and environmentally responsible analytical methods for the simultaneous quantification of antidiabetic drug combinations. Metformin hydrochloride (MET), a first-line biguanide antidiabetic agent, and Dapagliflozin (DAPA), a novel sodium-glucose cotransporter-2 (SGLT-2) inhibitor, represent a clinically significant FDC increasingly prescribed in T2DM management. This review critically examines the principles of green analytical chemistry (GAC) as applied to reversed-phase high-performance liquid chromatography (RP-HPLC) method development, with emphasis on the simultaneous estimation of MET and DAPA in pharmaceutical formulations. The review covers the pharmacological background of both drugs, physicochemical properties relevant to HPLC method design, green analytical chemistry principles and assessment tools (NEMI, GAPI, AGREE, Eco-Scale), chromatographic method development strategies, ICH Q2(R1) validation parameters, regulatory requirements, and a critical appraisal of published RP-HPLC methods for this drug combination. Emerging trends including ultra-high-performance liquid chromatography (UHPLC), green mobile phase alternatives, and multivariate optimization approaches are also

discussed. This review serves as a comprehensive reference for analytical scientists developing sustainable chromatographic methods for antidiabetic FDCs.

KEYWORDS: Green Analytical Chemistry, RP-HPLC, Metformin Hydrochloride, Dapagliflozin, Fixed-Dose Combination, ICH Q2(R1) Validation, SGLT-2 Inhibitor, Method Development, AGREE, NEMI.

1. INTRODUCTION

1.1 Global Burden of Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) represents one of the most significant non-communicable disease epidemics of the contemporary era. According to the International Diabetes Federation (IDF) Diabetes Atlas (10th Edition, 2021), approximately 537 million adults aged 20–79 years were living with diabetes globally, a figure projected to rise to 783 million by 2045. The associated economic burden exceeds USD 966 billion annually in healthcare expenditures, placing immense pressure on global health systems. India, with over 74 million diabetics, ranks as the second most affected country worldwide, making the development and quality control of antidiabetic medications of critical national and international importance.

The pharmacological management of T2DM has evolved substantially over the past two decades, shifting from monotherapy with metformin and sulfonylureas toward combination regimens targeting multiple pathophysiological mechanisms simultaneously. Fixed-dose combinations (FDCs) have gained widespread acceptance as they simplify dosing regimens, improve patient adherence, reduce pill burden, and potentially offer additive or synergistic therapeutic benefits. The combination of Metformin Hydrochloride with Dapagliflozin represents one such clinically validated FDC, combining complementary mechanisms of action to achieve superior glycemic control with cardiovascular and renal benefits.

1.2 Drug Profile: Metformin Hydrochloride

Metformin hydrochloride (MET; molecular formula: C4H11N5.HCl; molecular weight: 165.63 g/mol) is a biguanide oral antidiabetic agent and the cornerstone of T2DM pharmacotherapy worldwide. It primarily acts by inhibiting hepatic gluconeogenesis via activation of AMP-activated protein kinase (AMPK) and suppression of mitochondrial respiratory chain Complex I. Additionally, metformin enhances peripheral insulin sensitivity, reduces intestinal glucose absorption, and exerts anti-inflammatory effects. Metformin is freely soluble in water, practically insoluble in acetone, ether, and chloroform, with a pKa of

2.8 and 11.5, and exhibits UV absorption maxima at approximately 232 nm. Its high aqueous solubility and low lipophilicity (log P = -1.43) present distinct challenges in HPLC method development, often requiring ion-pairing reagents or specific column chemistries for adequate retention on reverse-phase columns.

1.3 Drug Profile: Dapagliflozin

Dapagliflozin (DAPA; molecular formula: C21H25ClO6; molecular weight: 408.87 g/mol) is a highly selective sodium-glucose cotransporter-2 (SGLT-2) inhibitor approved by the FDA in 2012 for the management of T2DM. SGLT-2, expressed predominantly in the renal proximal convoluted tubule (S1 segment), is responsible for approximately 90% of renal glucose reabsorption. Dapagliflozin inhibits SGLT-2 with high selectivity (>1200-fold over SGLT-1), promoting urinary glucose excretion (glucosuria) of 50–80 g/day, thereby reducing plasma glucose independent of insulin secretion or action. Beyond glycemic control, dapagliflozin demonstrates cardioprotective (DAPA-HF trial) and nephroprotective effects (DAPA-CKD trial), expanding its clinical utility. Dapagliflozin is practically insoluble in water, soluble in organic solvents, with log P of approximately 2.4 and UV absorption maxima at approximately 224 nm. Its hydrophobic character facilitates RP-HPLC analysis but requires optimization of mobile phase composition for simultaneous analysis with the highly polar metformin.

1.4 Rationale for Simultaneous Estimation

The co-formulation of MET and DAPA in a single dosage form as an FDC necessitates robust analytical methods capable of simultaneously quantifying both drugs with high specificity, accuracy, and precision. Analytical challenges inherent to this combination include the vast difference in polarity (MET: highly polar; DAPA: moderately lipophilic), different UV absorption maxima, and the potential for matrix interference from excipients. The development of a single isocratic or gradient RP-HPLC method capable of resolving both analytes with acceptable chromatographic parameters (resolution > 2.0, tailing factor < 2.0, theoretical plates > 2000) represents a significant analytical challenge that this review addresses comprehensively.

2. PRINCIPLES OF GREEN ANALYTICAL CHEMISTRY

2.1 Concept and Evolution

Green Analytical Chemistry (GAC) emerged as a paradigm shift in analytical sciences in the late 1990s, driven by growing awareness of the environmental, health, and safety impacts of

conventional analytical methodologies. Pioneered by Anastas and Warner's '12 Principles of Green Chemistry' (1998), the GAC framework was subsequently adapted by Namiesnik (2001) and further developed by Galuszka et al. into the '12 Principles of Green Analytical Chemistry' (PGAC). These principles advocate for the minimization or elimination of hazardous chemical usage, reduction of energy consumption, miniaturization of analytical procedures, online sample preparation, and replacement of toxic solvents with environmentally benign alternatives.

In chromatographic analysis, the transition toward greener methodologies has been driven by the replacement of hazardous organic solvents (acetonitrile, methanol, chloroform, n-hexane) with less toxic alternatives (ethanol, water, supercritical CO2), reduction of mobile phase consumption through flow rate optimization and column miniaturization, and the development of shorter analysis times that reduce overall energy and solvent consumption.

2.2 Green Analytical Chemistry Assessment Tools

Several standardized metrics have been developed to objectively assess and compare the environmental impact of analytical methods:

- NEMI (National Environmental Methods Index): A qualitative pictogram-based tool that evaluates methods based on four criteria — hazardous reagents, waste generated, corrosive reagents, and operator safety hazards. Methods meeting all four criteria receive a green circle designation, indicating high greenness.
- GAPI (Green Analytical Procedure Index): A more comprehensive semi-quantitative tool using a pentagram-divided diagram with 15 fields representing different stages of analytical procedure (sample collection, storage, transportation, preparation, measurement, waste generation). Each field is color-coded (green, yellow, or red) based on its environmental impact.
- AGREE (Analytical GREEnness metric): A recently developed holistic 12-criteria assessment tool that assigns numerical scores (0–1) to each criterion of the analytical procedure, producing an overall greenness score represented as a clock-face diagram. Criteria include sample throughput, miniaturization, reagent/solvent hazard, waste generation, operator safety, and energy consumption. Higher AGREE scores indicate greener methods.
- Eco-Scale: A penalty point-based system that deducts points from a maximum score of 100 based on hazardous reagents, waste generated, occupational hazard, and

instrumentation energy consumption. Methods scoring above 75 are classified as 'excellent green,' 50–75 as 'acceptable green,' and below 50 as 'inadequate.'

- AMVI (Analytical Method Volume Intensity): Focuses specifically on solvent consumption per analysis, providing a straightforward measure of solvent efficiency.

For the MET-DAPA FDC analytical method under review, AGREE and Eco-Scale assessments are recommended as primary greenness evaluation tools, given their comprehensive scope and quantitative output amenable to method comparison and reporting in regulatory submissions.

2.3 Green Mobile Phase Selection for MET-DAPA Analysis

The selection of appropriate mobile phase components is the most critical decision in green RP-HPLC method development. For simultaneous analysis of the polar MET and moderately lipophilic DAPA, the following green mobile phase strategies are considered:

- Ethanol-Water Systems: Ethanol (EtOH) is recognized as a 'preferred' green solvent and can partially replace methanol in mobile phases for RP-HPLC. EtOH-water mixtures with appropriate pH adjustment using green buffers (ammonium acetate, ammonium formate) can achieve adequate chromatographic resolution for MET-DAPA combinations.
- Methanol-Water Systems: While methanol carries some toxicity concerns, it is significantly more environmentally benign than acetonitrile (ACN) and is generally preferred over ACN in green HPLC method development. Isocratic methanol-water mobile phases with ammonium acetate buffer (pH 3.5–6.0) have demonstrated success in resolving this drug combination.
- Aqueous Buffer Optimization: Phosphate buffers, while effective, generate considerable aqueous waste with phosphate content. Green alternatives including ammonium acetate (volatile, compatible with LC-MS), ammonium formate, and citrate buffers are recommended. Volatile buffers additionally facilitate solvent recovery and waste treatment.
- pH Optimization: MET retention on C18 columns is highly pH-dependent due to its basic character (pKa 11.5). Mobile phases at pH 3.0–5.0 suppress ionization of basic excipients and improve peak shape. Ion-pair reagents (sodium dodecyl sulfate, heptafluorobutyric acid) can enhance MET retention without requiring extreme pH conditions.

3. RP-HPLC METHOD DEVELOPMENT STRATEGIES

3.1 Stationary Phase Selection

The stationary phase is a fundamental determinant of chromatographic selectivity for the MET-DAPA system. The following column chemistries have demonstrated suitability:

- C18 (Octadecylsilane, ODS) Columns: The most widely used RP-HPLC stationary phase, offering a broad range of selectivity through hydrophobic interactions. C18 columns with end-capping are preferred to minimize silanol activity and secondary interactions with the basic MET. Columns from reputed manufacturers (Waters Symmetry, Phenomenex Luna, Agilent Zorbax) with particle sizes of 3–5 micrometers and dimensions of 150 x 4.6 mm or 250 x 4.6 mm are commonly employed.
- C8 (Octylsilane) Columns: Shorter alkyl chains provide reduced hydrophobicity and may offer advantages for retaining the highly polar MET while maintaining adequate resolution from DAPA. C8 columns can facilitate the use of more aqueous mobile phases, contributing to greener methods.
- Polar-Embedded Phase Columns: Columns with embedded amide, carbamate, or ether functional groups (e.g., Waters XBridge Amide, Phenomenex Synergi Polar-RP) offer unique selectivity through mixed-mode interactions and can improve retention of polar analytes like MET under highly aqueous mobile phase conditions.
- HILIC (Hydrophilic Interaction Liquid Chromatography) Columns: While not strictly reverse-phase, HILIC stationary phases offer excellent retention for polar compounds like MET and represent an emerging green alternative due to their compatibility with high-organic mobile phases that require less energy for waste treatment.

3.2 Mobile Phase Optimization

Mobile phase optimization for simultaneous MET-DAPA analysis requires careful balancing of chromatographic resolution, analysis time, and greenness. Key optimization parameters include:

- Organic Modifier Ratio: Typical mobile phase compositions for MET-DAPA analysis range from 20:80 to 50:50 (v/v) organic modifier:aqueous buffer for isocratic conditions, with gradient elution offering greater flexibility for resolving the polarity-disparate analytes.
- Buffer pH: Mobile phase pH of 3.0–5.0 is optimal for MET-DAPA simultaneous analysis, suppressing ionization of MET (improving peak symmetry) while maintaining DAPA in its non-ionized, optimally retained form.

- Buffer Concentration: Ammonium acetate concentrations of 10–50 mM provide adequate ionic strength for stable retention while remaining compatible with green waste management practices and LC-MS compatibility for future method extension.
- Flow Rate: Optimized flow rates of 0.8–1.5 mL/min on conventional analytical columns balance analysis time with mobile phase consumption. Reduction to 0.3–0.5 mL/min on narrow-bore columns (2.1 mm ID) significantly reduces solvent consumption, contributing to higher greenness scores.
- Column Temperature: Elevated column temperatures (35–45°C) reduce mobile phase viscosity, improve mass transfer, lower back pressure, and can reduce organic modifier requirements, collectively contributing to greener operation.

3.3 UV Detection Wavelength Selection

Both MET and DAPA exhibit UV absorption in the range of 210–240 nm. MET shows maximum absorption at approximately 232 nm, while DAPA absorbs maximally at approximately 224 nm. A detection wavelength of 228–232 nm typically provides a satisfactory compromise for simultaneous UV detection of both analytes with adequate sensitivity. Photodiode array (PDA/DAD) detection is strongly recommended to provide spectral confirmation of peak purity and identity during method validation, ensuring specificity in the presence of potential degradation products and excipient interferences.

4. ICH Q2 (R1) METHOD VALIDATION PARAMETERS

Method validation according to ICH Q2(R1) guidelines is a regulatory requirement that ensures the suitability, reliability, and reproducibility of the analytical method for its intended purpose. The following parameters are validated for simultaneous RP-HPLC determination of MET and DAPA:

4.1 Specificity and Selectivity

Specificity is the ability of the method to unequivocally assess the analyte in the presence of other components including impurities, degradation products, and formulation excipients. For MET-DAPA FDC analysis, specificity is demonstrated by: (a) absence of interference from common tablet excipients (microcrystalline cellulose, lactose, magnesium stearate, HPMC) in the retention time windows of MET and DAPA; (b) peak purity assessment by PDA detector confirming homogeneous UV spectrum across each analyte peak; and (c) forced degradation studies demonstrating resolution of MET and DAPA from their degradation products under

acidic (0.1 N HCl), alkaline (0.1 N NaOH), oxidative (3% H2O2), photolytic (ICH Q1B conditions), and thermal (60°C, 5 days) stress conditions.

4.2 Linearity and Range

Linearity is established by analyzing a minimum of five concentration levels spanning 50–150% of the nominal analytical concentration. For MET, typical linearity ranges of 10–100 microg/mL are studied, while for DAPA, ranges of 1–20 microg/mL are appropriate reflecting their typical dosage ratio (500 mg MET : 10 mg DAPA in commercial FDCs). Correlation coefficients (r2) of >= 0.999 are required, and Y-intercepts should not significantly differ from zero. Residual plots and ANOVA for linearity are reported to confirm the linear relationship.

4.3 Accuracy (Recovery Studies)

Accuracy is assessed by standard addition method at three concentration levels (50%, 100%, 150% of nominal concentration) in triplicate (n=3). Percentage recovery values of 98.0–102.0% are considered acceptable for pharmaceutical analysis per ICH Q2(R1). For MET-DAPA FDC formulations, recovery studies are conducted by spiking known amounts of pure drug standards into placebo mixture containing all formulation excipients, thereby assessing the complete matrix effect of the formulation.

4.4 Precision

Precision is expressed as percent Relative Standard Deviation (%RSD) and evaluated at three levels:

- Repeatability (Intra-day Precision): Six replicate injections of three concentration levels on the same day by the same analyst using the same instrument. %RSD <= 2.0% is required.
- Intermediate Precision (Inter-day Precision): Analysis performed on three consecutive days by different analysts using different instruments/columns. %RSD <= 2.0% is required.
- Reproducibility: Not always reported in method development papers but expected for methods intended for multi-laboratory or multi-site use. Transfer between different laboratories should yield %RSD <= 2.0%.

4.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ are determined by the signal-to-noise (S/N) ratio method — LOD at S/N = 3:1 and LOQ at S/N = 10:1 — or by calculation from the standard deviation of response and slope of the calibration curve: LOD = 3.3 sigma/S; LOQ = 10 sigma/S. Published methods typically report LOD values of 0.1–0.5 microg/mL and LOQ values of 0.3–1.5 microg/mL for MET in this combination. For DAPA, LOD values of 0.05–0.2 microg/mL and LOQ values of 0.15–0.6 microg/mL are typical.

4.6 Robustness

Robustness evaluates the capacity of the method to remain unaffected by small but deliberate variations in method parameters, providing an indication of reliability during normal usage. Parameters typically varied for robustness testing of MET-DAPA HPLC methods include: flow rate (±0.2 mL/min), mobile phase pH (±0.2 units), mobile phase organic composition (±2%), column temperature (±5°C), and column brand/lot variation. Robustness is expressed as %RSD of chromatographic responses, with acceptable limits of <= 2.0%. Plackett-Burman experimental design is increasingly recommended for simultaneous evaluation of multiple robustness factors, as it provides statistical rigor with minimal experimental runs.

4.7 System Suitability Testing

System suitability tests (SST) verify that the chromatographic system is performing adequately prior to sample analysis. Standard SST parameters for MET-DAPA RP-HPLC methods include:

Parameter	MET — Acceptance Criteria	DAPA — Acceptance Criteria
Theoretical Plates (N)	>= 2000	>= 2000
Tailing Factor (T)	<= 2.0	<= 2.0
Resolution (Rs)	>= 2.0 (from DAPA)	>= 2.0 (from MET)
Retention Factor (k')	>= 1.0	>= 1.0
% RSD (n=6 injections)	<= 2.0%	<= 2.0%

5. CRITICAL REVIEW OF PUBLISHED ANALYTICAL METHODS

5.1 UV Spectrophotometric Methods

Before the widespread adoption of HPLC methods, UV spectrophotometry was the primary technique for analysis of MET and DAPA. Simultaneous UV estimation of this combination is challenged by spectral overlap, particularly in the 220–240 nm region. Chemometric approaches including simultaneous equations, Q-absorbance, first-derivative spectrophotometry, and multivariate calibration (PCR, PLS) have been applied to resolve

spectral overlap and achieve simultaneous quantification. While UV methods offer simplicity and low instrumentation cost, they lack the specificity and sensitivity achievable by HPLC, particularly in the presence of degradation products and excipient interferences.

5.2 RP-HPLC Methods

Multiple RP-HPLC methods have been reported for simultaneous estimation of MET and DAPA in pharmaceutical formulations. A critical summary of representative methods includes:

- C18 Column, Methanol:Ammonium Acetate Buffer (pH 4.5) — 60:40 v/v, Flow Rate: 1.0 mL/min, UV Detection at 228 nm: Achieves baseline resolution with retention times of approximately 2.8 min (MET) and 6.4 min (DAPA). Linear range: MET 10–100 microg/mL; DAPA 1–10 microg/mL. AGREE score: 0.61, indicating acceptable greenness due to methanol use and moderate solvent consumption.
- C18 Column, Acetonitrile:Water (0.1% Formic Acid) — 30:70 v/v, Flow Rate: 1.2 mL/min, UV Detection at 230 nm: Provides good resolution (Rs > 3.5) with shorter analysis time (~8 min total). NEMI assessment indicates non-green classification due to ACN and formic acid usage. AGREE score: 0.55.
- C8 Column, Methanol:Phosphate Buffer (pH 4.0) — 40:60 v/v, Flow Rate: 0.8 mL/min, UV Detection at 232 nm: Demonstrates improved MET retention on C8 phase. Robustness evaluated by Plackett-Burman design. Eco-Scale score: 68 (acceptable green). Recovery: 99.1–101.4% for both analytes.
- Core-Shell C18 Column (Kinetex), Ethanol:Ammonium Formate Buffer (pH 5.0) — 35:65 v/v, Flow Rate: 0.6 mL/min, UV Detection at 228 nm: A genuinely green method employing ethanol as organic modifier and volatile ammonium formate buffer. Analysis time < 6 min. AGREE score: 0.74, Eco-Scale: 79 (excellent green). Demonstrates that truly green HPLC methods for this combination are achievable.

A consistent observation across reported methods is the inverse relationship between analysis speed and greenness — shorter analysis times generally require higher organic modifier concentrations or flow rates, increasing solvent consumption per unit time. Optimization through column miniaturization (2.1 mm ID columns, sub-2 micron particles) offers the most promising strategy for achieving both rapid analysis and high greenness simultaneously.

5.3 UHPLC Methods

Ultra-high-performance liquid chromatography (UHPLC), utilizing sub-2 micron particle columns operated at elevated pressures (>6000 psi), offers significantly reduced analysis times (2–4 min vs. 8–15 min for conventional HPLC) and dramatically reduced solvent consumption (3–10-fold reduction) with equivalent or superior resolution. UHPLC methods for MET-DAPA estimation using BEH C18 (1.7 microm, 50 x 2.1 mm) columns with methanol:ammonium acetate buffer mobile phases achieve complete baseline resolution within 3.5 minutes while consuming approximately 80% less mobile phase compared to conventional HPLC methods, substantially improving environmental sustainability.

6. FORCED DEGRADATION AND STABILITY STUDIES

Stress testing or forced degradation studies are conducted to characterize the stability-indicating capability of the analytical method, identify potential degradation pathways, and elucidate the intrinsic stability of MET and DAPA under various environmental conditions. ICH Q1A(R2) guidelines govern the design of stability studies for pharmaceutical products.

- at typical storage temperatures, with degradation levels below 2% under forced thermal conditions, indicating adequate stability in solid dosage forms under appropriate storage conditions.

7. APPLICATION TO PHARMACEUTICAL FORMULATION ANALYSIS

7.1 Sample Preparation for Tablet Analysis

Sample preparation for MET-DAPA FDC tablet analysis involves: (a) weighing and powdering a defined number of tablets (typically 20) to obtain a homogeneous powder; (b) accurately weighing a portion equivalent to the label claim of both drugs; (c) transferring to a volumetric flask with approximately 50 mL of mobile phase or diluent; (d) sonicating for 15–20 minutes with intermittent shaking to ensure complete dissolution; (e) cooling to room temperature and diluting to volume; (f) filtering through a 0.45 micrometer nylon or PVDF membrane filter, discarding the first 5 mL of filtrate; and (g) further diluting aliquots of the filtrate to obtain concentrations within the linearity range prior to injection.

Green sample preparation modifications include minimizing extraction solvent volumes, using aqueous or ethanolic extraction instead of acetonitrile-based systems, and employing direct dissolution methods that eliminate organic solvent extraction steps wherever chromatographic resolution permits.

7.2 Assay Results

Validated green RP-HPLC methods report label claim assay values of 98.5–101.8% for MET and 98.2–101.5% for DAPA in commercial Metformin HCl (500 mg) + Dapagliflozin (10 mg) FDC tablets. These values are within the pharmacopoeial acceptance criteria of 98.0–102.0%, confirming the analytical accuracy and suitability of validated methods for routine quality control of commercial FDC formulations.

8. REGULATORY PERSPECTIVES

The regulatory landscape for analytical method development and validation for FDC formulations is governed by multiple guidelines and pharmacopoeial standards:

- ICH Q2(R1): The foundational guideline for validation of analytical procedures, defining methodology and acceptance criteria for all key validation parameters discussed in Section 4.
- ICH Q1A(R2): Governs stability testing of new drug substances and products, providing the framework for forced degradation and long-term stability study designs.
- ICH Q3B(R2): Addresses degradation products in new drug products, guiding the identification and qualification thresholds for degradation products detected by stability-indicating methods.
- USP <1225>: United States Pharmacopeia general chapter on validation of compendial procedures, providing additional guidance on validation methodology.
- WHO Technical Report Series (TRS 992, Annex 6): WHO guidelines on analytical method validation for pharmaceutical quality control, particularly relevant for developing country regulatory submissions.
- CDSCO Guidelines (India): Central Drugs Standard Control Organization requirements for analytical method validation in Indian regulatory submissions, requiring methods validated per ICH Q2(R1) with additional requirements for local stability studies.

Green analytical methods, while not explicitly mandated by current ICH guidelines, are increasingly encouraged by regulatory agencies as part of sustainability initiatives. The European Medicines Agency (EMA) has incorporated sustainability considerations into its environmental risk assessment framework, and the FDA's Pharmaceutical Quality/CMC initiative increasingly encourages green manufacturing and analytical practices.

9. EMERGING TRENDS AND FUTURE DIRECTIONS

- Supercritical Fluid Chromatography (SFC): CO2-based mobile phases in SFC offer exceptional greenness and are emerging as viable alternatives to RP-HPLC for pharmaceutical analysis. SFC methods for MET-DAPA analysis are anticipated given the success of SFC for other antidiabetic drug combinations.
- Micellar Liquid Chromatography (MLC): Using surfactant solutions (SDS, Brij-35) as mobile phases eliminates organic solvent use almost entirely, offering maximal greenness. MLC methods for MET and related polar drugs have been reported and may be extended to DAPA combinations.
- Multivariate Optimization (DoE): Design of Experiments approaches including Box-Behnken, Central Composite, and D-optimal designs enable simultaneous optimization of multiple chromatographic variables with minimal experimentation, producing statistically robust optimized methods.
- Quality by Design (QbD) Approach: The ICH Q8(R2) QbD framework is increasingly applied to analytical method development, defining analytical target profiles (ATP) and method operable design regions (MODR) to build quality and understanding directly into method development.
- Machine Learning and AI in Method Development: Artificial intelligence and machine learning algorithms are being explored for prediction of optimal HPLC conditions (column type, mobile phase composition, gradient profile) based on molecular descriptors of analytes, potentially transforming the trial-and-error approach to a rational, data-driven method development paradigm.
- Nano-HPLC and Capillary HPLC: Sub-microliter injection volumes and nano-scale columns dramatically reduce solvent consumption and enable analysis of limited sample volumes, representing the ultimate expression of green miniaturization in chromatographic analysis.

10. CONCLUSION

The simultaneous estimation of Metformin Hydrochloride and Dapagliflozin in fixed-dose combination formulations represents an analytically challenging but pharmaceutically essential task. The divergent physicochemical properties of the two analytes — highly polar MET and moderately lipophilic DAPA — demand careful chromatographic optimization to achieve simultaneous resolution, sensitivity, and accuracy.

This review has comprehensively examined the pharmacological rationale for the MET-DAPA FDC, the theoretical and practical aspects of green RP-HPLC method development, ICH Q2(R1) validation requirements, critical appraisal of published methods, and emerging analytical trends. The integration of Green Analytical Chemistry principles into RP-HPLC method development for this drug combination is not merely an ethical imperative but an increasingly practical reality, demonstrated by methods employing ethanol-based mobile phases with volatile buffers that achieve AGREE scores of 0.70+ while maintaining full ICH-compliant validation performance.

The development of a truly green, fully validated, stability-indicating RP-HPLC-UV method for simultaneous estimation of MET and DAPA represents a meaningful contribution to pharmaceutical analytical science. Such methods, assessed by rigorous greenness metrics including AGREE, NEMI, GAPI, and Eco-Scale, and validated comprehensively per ICH Q2(R1) with forced degradation studies, will serve both the scientific community and the regulatory environment as sustainable analytical tools for quality control of this increasingly important antidiabetic FDC. Future directions including UHPLC, SFC, and AI-assisted method development hold great promise for further advancing the greenness, speed, and robustness of chromatographic methods in pharmaceutical analysis.

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