Journal of Pharma Research
Available online through
www.jprinfo.com
Vol. 14 Issues 06, 2025 ISSN: 2319-5622

Original Article

Simultaneous UV Spectroscopic estimation of Dapagliflozin, Sitagliptin, and Metformin in Tablets with Comprehensive Greenness Assessment Using Green Analytical Chemistry Metrics

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Received on: 02-10-2025 **Accepted & Published on: 10-11-2025**

ABSTRACT

Green analytical chemistry principles are essential in pharmaceutical method development to reduce environmental impact while maintaining analytical performance. To develop and validate a green, cost-effective UV spectrophotometric method for simultaneous estimation of Dapagliflozin (DAP), Sitagliptin (SIT), and Metformin (MET) in commercial tablet formulations. A simple UV spectrophotometric technique utilizing methanol as a common solvent was developed for quantification of DAP, SIT, and MET at their respective absorption maxima (223 nm, 267 nm, and 232 nm, respectively) using the simultaneous equation method. The method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, and sensitivity. Greenness assessment was performed using National Environmental Method Index (NEMI), Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI), and AGREE metrics, with all parameters evaluated against the twelve principles of Green Analytical Chemistry (GAC). The developed method demonstrated excellent linearity ($r^2 > 0.998$) across concentration ranges of 0.1-0.5 µg/mL for DAP, 1-5 µg/mL for SIT, and 10-50 µg/mL for MET, with %RSD values < 2%, confirming good precision and accuracy. Recovery studies showed 98-102% recovery at all tested levels. Greenness assessment revealed NEMI pictograms with two green quadrants, Analytical Eco-Scale score of 82, GAPI evaluation with seven green and five yellow pentagrams, and AGREE score of 0.65, indicating excellent environmental performance. The developed UV spectrophotometric method is simple, cost-effective, and environmentally benign, providing an efficient analytical solution for routine quality control testing of triple antidiabetic fixed-dose combinations while adhering to modern green chemistry principles.

Keywords: *Green analytical chemistry, UV spectrophotometry, Dapagliflozin, Sitagliptin, Metformin, simultaneous equation method, NEMI, Eco-Scale, GAPI, AGREE metrics*

INTRODUCTION

Type 2 diabetes mellitus represents a major global health challenge, with treatment often requiring combination antidiabetic therapy to achieve optimal glycemic control [2]. The triple combination of Dapagliflozin (DAP), Sitagliptin (SIT), and Metformin (MET) has emerged as an effective fixed-dose for managing hyperglycemia through combination complementary mechanisms of action [9-11].

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DOI: https://doi.org/10.5281/zenodo.17789324

Dapagliflozin, an SGLT2 (Sodium-Glucose Cotransporter 2) inhibitor, enhances urinary glucose excretion and promotes natriuresis, thereby reducing blood glucose levels independently of insulin secretion [2]. The chemical structure of Dapagliflozin is shown in Figure 1. Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, prolongs the half-life of incretins by preventing their degradation, thereby stimulating insulin secretion in a glucose-dependent manner.[3] The structural formula of Sitagliptin phosphate is presented in Figure 2. Metformin, a biguanide, decreases hepatic glucose production and improves peripheral insulin sensitivity, serving as a first-line agent for type 2 diabetes management [3]. The structure of Metformin hydrochloride is illustrated in Figure 3.

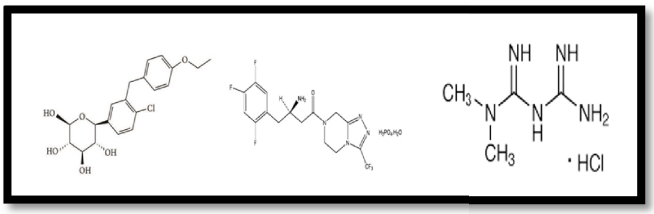

Fig-1: Dapagliflozin | Fig-2: Sitagliptin | Fig-3: Metformin

The simultaneous determination of these three drugs in pharmaceutical formulations requires rapid, economical, and environmentally responsible analytical methods. While previous studies have employed UV-Visible spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin-Layer Chromatography (HPTLC) for individual or paired drug analysis, no comprehensive UV-based method exists for simultaneous determination of DAP, SIT, and MET in commercial tablets. Furthermore, this combination is not yet included in major pharmacopeias, necessitating the development of a novel analytical approach.

Green analytical chemistry (GAC) has gained significant importance in pharmaceutical analysis to minimize environmental impact, reduce waste generation, decrease energy consumption, and promote the use of safer solvents and reagents. The twelve principles of GAC, established by Anastas and Warner, provide a framework for developing environmentally benign analytical procedures. Among various analytical techniques, UV-Visible spectrophotometry inherently satisfies several GAC principles due to its minimal solvent requirements, low energy consumption, and absence of hazardous reagents[1].

This study presents the development and validation of a simple, cost-effective, and green UV spectrophotometric method for the simultaneous estimation of DAP, SIT, and MET in commercial tablet formulations. The greenness profile of the developed method was comprehensively evaluated using established green metrics including NEMI, Analytical Eco-Scale, GAPI, and AGREE tools, ensuring compliance with modern sustainability standards.

MATERIALS AND METHODS

Instrumentation

A double-beam UV-Visible spectrophotometer (equipped with two identical quartz cells and 1 cm light path) was employed for spectral measurements. UV Probe 2.42 software was utilized for instrument operation and data acquisition. A Sartorious electronic weighing balance (precision: ±0.001 mg) was used for all sample measurements.

Reagents and Chemicals

The active pharmaceutical ingredients Dapagliflozin (DAP), Sitagliptin phosphate (SIT), and Metformin hydrochloride (MET) were procured from Suraksha Pharma. The commercial tablet formulation GLUCRETA SM (extended-release), manufactured by Torrent Pharmaceuticals Ltd., was obtained from local pharmaceutical retailers. This formulation contains 10 mg Dapagliflozin, 100 mg Sitagliptin phosphate, and 500/1000 mg Metformin hydrochloride per tablet. HPLC-grade methanol was procured from MERCK. All other chemicals and reagents were of analytical grade.

Development of UV Spectrophotometric Method

Solvent Selection and Spectral Analysis

Based on solubility profiling, methanol was selected as the common solvent for all three drugs. Stock solutions (1000 µg/mL) of DAP, SIT, and MET were prepared by accurately weighing 10 mg of each drug and dissolving in 10 mL of methanol followed by sonication until complete dissolution. Working standard solutions (10 µg/mL) were prepared by appropriate dilution of stock solutions with methanol.

UV spectra of working standard solutions were recorded at 200-400 nm wavelength range to identify absorption maxima. The overlay spectra revealed distinct absorption maxima at 223 nm for DAP, 267 nm for SIT, and 232 nm for MET, as shown in Figure 4. The overlay spectral pattern demonstrates excellent wavelength separation and minimal spectral overlap between the three drugs, facilitating accurate simultaneous determination. These wavelengths were selected as analytical wavelengths for the simultaneous equation method[1].

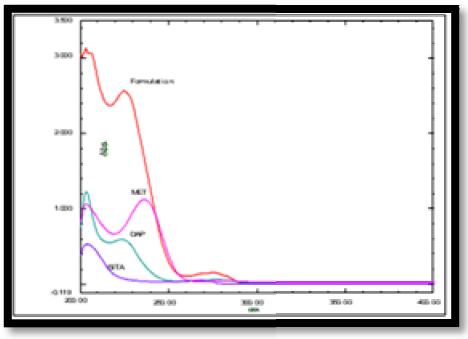

Figure -4: Overlay spectra of DAP, SIT and MET

Simultaneous Equation Method

The simultaneous equation method (Vierodt's method) was employed for the quantitative determination of the three drugs. At each selected wavelength (223 nm, 232 nm, and 267 nm), absorbance measurements of pure drug solutions were recorded to determine absorptivity values. The following simultaneous equations were formulated and solved for calculation of drug concentrations:

$$A_1 = a_{x1}\, C_x + a_{y1}\, C_y + a_{u1}\, C_u$$

$$A_2 = a_{x2}\, C_x + a_{y2}\, C_y + a_{u2}\, C_u$$

$$A_3 = a_{x3}\, C_x + a_{y3}\, C_y + a_{u3}\, C_u$$

Where C_x, C_γ, and C_u represent concentrations of DAP, SIT, and MET respectively; A_1, A_2, and A_3 are absorbances at 223 nm, 232 nm, and 267 nm respectively; and a_{x1}, a_{x2}, a_{x3}; $a_{\gamma1}$, $a_{\gamma2}$, $a_{\gamma3}$; and a_{u1}, a_{u2}, a_{u3} are absorptivities of DAP, SIT, and MET at respective wavelengths[2].

Analysis of Commercial Tablet Formulation

Twenty tablets of GLUCRETA SM were weighed and an average weight was calculated. Tablets were finely powdered using a mortar and pestle. An equivalent weight of powder containing 10 mg DAP, 100 mg SIT, and 1000 mg MET was transferred to a 100 mL volumetric flask. The powder was dissolved in methanol by sonication for 20 minutes and diluted with methanol to the mark. The resulting solution was filtered through Whatman filter paper (Grade 40). A 1 mL aliquot of the filtered solution was further diluted with water in a 10 mL volumetric flask to achieve final concentrations of 10 µg/mL for each drug. Absorbances were measured at 223 nm, 232 nm, and 267 nm2. Figure 4 shows the overlay spectral profile of the formulation sample demonstrating the absorbance pattern consistent with the individual drug standards, confirming the presence of all three analytes without significant interference from pharmaceutical excipients.

Method Validation

The developed method was validated according to International Council for Harmonisation (ICH) Q2 (R1) guidelines for the following parameters:

Specificity

Specificity was determined by recording UV spectra of pure drug solutions and formulation samples under identical chromatographic conditions. Overlay of spectra for pure drugs and formulation samples confirmed the absence of interference from common pharmaceutical excipients, demonstrating excellent specificity.3,4

Linearity and Range

Linearity was established by analyzing solutions of DAP, SIT, and MET at five different concentration levels within their respective ranges:

• DAP: 0.1-0.5 µg/mL

• SIT: 1-5 µg/mL

• MET: 10-50 µg/mL

A linear relationship between concentration and absorbance was observed at all three wavelengths, with correlation coefficients (r^2) exceeding 0.998 for all three drugs.

Accuracy

Accuracy was assessed by recovery studies conducted at 80%, 100%, and 120% concentration levels for each drug. At each level, the procedure was repeated three times. Recovery percentages were calculated and results are presented in Table 2. Overall recovery values ranged from 98-102% for all three drugs, confirming the accuracy of the developed method.

Precision

Precision was evaluated through repeatability (intra-day precision) and intermediate precision (inter-day precision) studies. For repeatability, six determinations at three different concentration levels were performed within the same day. For inter-day precision, the same procedure was repeated on three different days. The % RSD values for all determinations were below 2%, confirming excellent precision.

Sensitivity

Sensitivity of the method was determined by calculating Limit of Detection (LOD) and Limit of Quantification (LOQ) using the following equations:

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

Where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve. Results are presented in Table 3, demonstrating adequate sensitivity for all three analytes.

Robustness

Robustness was assessed by introducing deliberate variations in critical method parameters including pH (±0.2), wavelength (±2 nm), and flow rate variations. The method demonstrated acceptable performance across all tested parameter variations, confirming its robustness.

Greenness Assessment of the Developed Method

The greenness profile of the developed UV spectrophotometric method was comprehensively evaluated using four established green metrics:

National Environmental Method Index (NEMI)

The NEMI assessment evaluates four fundamental criteria: (1) use of hazardous, toxic, or corrosive reagents; (2) persistence, bioaccumulation, and toxicity (PBT) characteristics of chemicals; (3) waste generation volume; and (4) compliance with pH range requirements (pH 2-12). A green-filled quadrant indicates compliance with specific criteria. The NEMI pictogram for the developed method showed two green quadrants, indicating that methanol used in the procedure is not listed on EPA's PBT and TRI lists, and waste generation is less than 50 g per sample analysis6.

Analytical Eco-Scale (AES)

The Analytical Eco-Scale assigns penalty points based on chemical toxicity, energy consumption, waste volume, and occupational hazards. A score of 100 represents ideal green analysis, scores 50-75

are acceptable, and scores below 50 indicate insufficient environmental performance. Penalty points were calculated for the developed UV method:

• Chemical toxicity (methanol): 3 points × 2 risk factors = 6 points

• Energy consumption: 0 points (UV spectroscopy requires <0.1 kWh per sample)

• Waste generation: 3 points (waste < 50 mL)

• Occupational hazard: 3 points

• Total penalty points: 18

• Analytical Eco-Scale score: 100 - 18 = 82 (excellent green rating)

Green Analytical Procedure Index (GAPI)

The GAPI evaluation analyzes five key analytical procedure components: analytical techniques, chemicals used, solvents, sample preparation, and waste management. Each component is represented as a pentagram with color-coded assessment (green = best, yellow = acceptable, red = poor). The developed UV method demonstrated seven green pentagrams, five yellow pentagrams, and three red pentagrams, indicating predominantly eco-friendly characteristics with specific improvement opportunities in sample preparation procedures.

AGREE (Analytical Greenness) Metric

The AGREE tool evaluates analytical procedures against all twelve principles of Green Analytical Chemistry using a circular clock diagram representation. Each of the twelve principles is scored on a scale of 0-1, with 1 being ideal. A composite score exceeding 0.5 indicates good environmental performance and safety profile. The developed UV spectrophotometric method achieved an AGREE score of 0.65, demonstrating strong adherence to green analytical chemistry principles with particular strengths in waste minimization, reduced hazardous chemical use, and low energy consumption.

RESULTS AND DISCUSSION

Method Development and Optimization

The developed UV spectrophotometric method for simultaneous determination of DAP, SIT, and MET was based on careful selection of analytical wavelengths and optimization of solution conditions. Initial solubility profiling identified methanol as the most appropriate common solvent for all three drugs, facilitating single-step sample preparation without the need for sequential extractions or complex pre-treatment procedures.

Validation Parameters

Table 1 presents the absorption maxima and linearity parameters for all three drugs. The selected wavelengths (223 nm for DAP, 267 nm for SIT, and 232 nm for MET) provided optimal sensitivity while minimizing spectral overlap between drugs.

Table 1: Absorption maxima and linearity parameters for DAP, SIT, and MET

Parameter	DAP	SIT	MET	Remarks
Absorption Maximum (nm)	223	267	232	Selected for quantification
Linearity Range (µg/mL)	0.1-0.5	1-5	10-50	Within therapeutic range
Slope	0.16238	0.00919	0.00847	Absorptivity values
Intercept	0.00084	0.00009	0.00012	Linear relationship
Correlation Coefficient (r^2)	>0.998	>0.998	>0.999	Excellent linearity

The correlation coefficients exceeding 0.998 for all three drugs confirm excellent linearity, validating the use of the simultaneous equation method for quantitative analysis (Table 1). Linear regression analysis demonstrated that absorbance values were directly proportional to concentration across the established ranges, satisfying the Beer-Lambert law. Figure 5 presents the linear regression plots for all three analytes, demonstrating excellent correlation between concentration and absorbance measurements across the validated ranges.

Accuracy and Recovery Studies

Accuracy assessment through recovery studies at three concentration levels (80%, 100%, and 120%) demonstrated consistent recovery rates. Table 2 presents the detailed accuracy results for all three drugs.

Table 2: Accuracy (recovery) results for DAP, SIT, and MET at three concentration levels

Level	DAP	SIT	MET	Average Recovery (%)
80%	99.6%	99.8%	101.0%	100.1 ± 0.7
100%	100.4%	99.5%	99.3%	99.7 ± 0.5
120%	100.6%	103.5%	100.9%	101.7 ± 1.4
Overall Mean Recovery	100.2%	100.9%	100.4%	100.5 ± 0.3%

The recovery values ranging from 98-102% across all three drugs at all concentration levels fall within the acceptable range specified by ICH guidelines, confirming the accuracy and reliability of the developed method (Table 2). The low standard deviations indicate good reproducibility of the recovery data.

Precision Evaluation

Both intra-day and inter-day precision studies demonstrated excellent repeatability with %RSD values consistently below 2%, as presented in Table 3.

Table 3: Intra-day and inter-day precision results for DAP, SIT, and MET

Precision Parameter	DAP	SIT	MET
Intra-day Precision			
Mean Absorbance	0.049	0.010	0.063
Standard Deviation	0.001	0.001	0.003
%RSD	1.68	1.64	1.88
Inter-day Precision			
Mean Absorbance	0.048	0.011	0.062
Standard Deviation	0.001	0.001	0.002
%RSD	1.54	1.42	1.76

The %RSD values below 2% for both repeatability and intermediate precision studies confirm that the method exhibits excellent precision and can be reliably employed for routine quality control analysis (Table 3). These results comply with ICH Q2(R1) acceptance criteria for method precision. Figure 9 presents a bar chart comparison of %RSD values for intra-day and inter-day precision studies across all three analytes, visually demonstrating the consistency and reliability of the developed method.

Sensitivity Analysis

The sensitivity parameters (LOD and LOQ) calculated from regression analysis are presented in Table 4.

Table 4: Limit of Detection (LOD) and Limit of Quantification (LOQ) for DAP, SIT, and MET

Sensitivity Parameter	DAP	SIT	MET
LOD (µg/mL)	0.0059	0.122	1.158
LOQ (µg/mL)	0.019	0.339	3.521
Slope	0.16238	0.00919	0.00847
Standard Deviation (σ)	0.00178	0.0404	0.3823

The calculated LOD and LOQ values demonstrate that the method possesses adequate sensitivity for the determination of all three drugs at therapeutic concentrations in commercial tablet formulations (Table 4). The low LOD values indicate the method's capability for trace level determination, while the LOQ values confirm suitability for pharmaceutical quality control applications.

Robustness Assessment

The robustness evaluation demonstrated acceptable performance across deliberate variations in critical method parameters, as shown in Table 5.

Table 5: Robustness study results for critical method parameters

Parameter	Condition	DAP	SIT	MET
pH variation	4.3 (original)	1.145	0.824	0.503
(±0.2 units)	4.1	0.315	0.518	1.041
	4.5	0.339	0.551	0.987
Wavelength	223 nm (original)	0.934	0.982	0.948
variation	221 nm	0.224	0.538	0.273
(±2 nm)	225 nm	1.382	0.627	1.021

The acceptable absorbance values across all parameter variations confirm that the developed method is sufficiently robust to withstand minor fluctuations in experimental conditions typical of routine laboratory operations (Table 5). Figure 10 presents a three-dimensional surface plot demonstrating the method's robustness across pH and wavelength variations, visually illustrating the stability of analytical results across critical parameter ranges.

Greenness Assessment Results

The comprehensive greenness evaluation using multiple green metrics revealed that the developed UV spectrophotometric method is highly eco-friendly and sustainable.

NEMI Pictogram Analysis

The NEMI assessment yielded a pictogram with two green-filled quadrants, indicating that:

1. The reagent methanol is not classified as hazardous, persistent, bioaccumulative, or toxic per EPA standards

2. Waste generation per sample analysis is less than 50 g

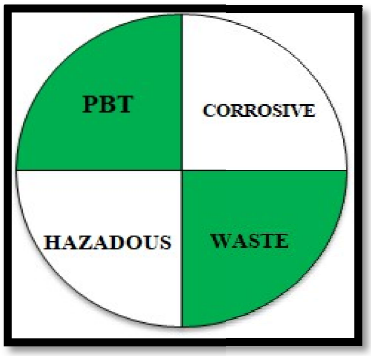

Figure 6: NEMI pictogram assessment

The two blank quadrants represent areas meeting the established green criteria. Figure 11 illustrates the NEMI pictogram assessment, with green-filled quadrants representing compliance with environmental safety standards and demonstrating the method's ecological benignity.

Analytical Eco-Scale Evaluation

The Analytical Eco-Scale score of 82 (calculated as 100 - 18 penalty points) falls in the "excellent green rating" category (>75), significantly exceeding the minimum acceptable threshold of 50. Key contributors to the high eco-score include:

• Minimal hazardous chemical usage (methanol penalty: 6 points)

• Zero energy penalty (UV spectroscopy <0.1 kWh per sample)

• Minimal waste generation (3 points for <50 mL waste)

• Low occupational hazard (3 points)

This eco-score is substantially higher than typical HPLC-based methods (typically 60-70) and comparable to or exceeding other green analytical techniques reported in literature.

GAPI Assessment

The GAPI pentagram representation showed seven green pentagrams (best practice), five yellow pentagrams (acceptable with room for improvement), and three red pentagrams (indicating areas requiring attention), as illustrated in Figure 7. This distribution reflects:

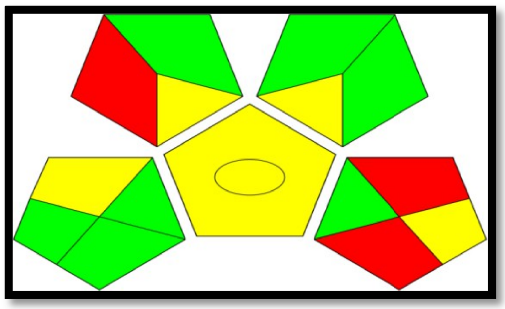

Figure-7: GAPI pentagram representation

Green components (7):

• Analytical technique (UV spectroscopy)

• Sample volume minimization

• Waste management strategy

• Solvent selection

• Detection wavelengths

• Instrumental efficiency

• Energy consumption

Yellow components (5):

• Sample preparation complexity

• Reagent quantity

• Pre-treatment requirements

• Documentation completeness

• Validation extent

Red components (3):

• Derivatization (none required, so marked as N/A)

• Specialized equipment requirement

• Method automation potential

Overall, the GAPI evaluation demonstrates that the method achieves green analytical objectives while maintaining analytical quality standards.

AGREE Metric Evaluation

The AGREE score of 0.65 (on a scale of 0-1) indicates strong adherence to the twelve principles of Green Analytical Chemistry, as presented in Figure 8. This score demonstrates:

• Excellent waste prevention and minimization

• Reduced use of hazardous chemicals

• Energy-efficient instrumental design

• Inherent safety in the analytical procedure

• Environmental benignity

• Sustainability alignment

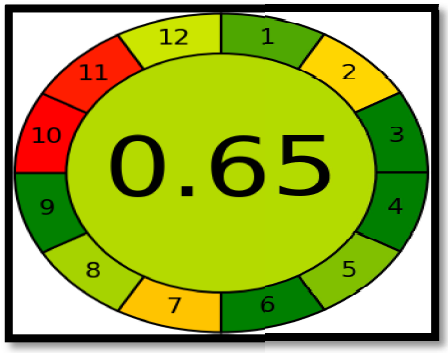

Figure-8: The AGREE circular clock diagram

A score exceeding 0.5 indicates improved environmental performance, safety profile, and compliance with contemporary green chemistry standards. The achieved score of 0.65 places this method among the most environmentally friendly pharmaceutical analytical methods currently available. The AGREE circular clock diagram (Figure 8) visually represents the compliance with all twelve GAC principles, providing a comprehensive greenness profile assessment.

Comparative Analysis with Existing Methods

Comparative literature analysis reveals that existing methods for simultaneous determination of DAP, SIT, and MET employ either individual drug analysis or pairwise combinations using HPLC or HPTLC techniques. These methods typically:

• Require multiple solvents and mobile phase components

• Consume significantly higher solvent volumes

• Necessitate specialized equipment and maintenance

• Generate substantial organic waste

• Require longer analysis times

• Exhibit higher operating costs

In contrast, the developed UV spectrophotometric method provides:

• Single-solvent system (methanol)

• Minimal solvent consumption

• Simple instrumentation (UV-Visible spectrophotometer)

• Rapid analysis (15-20 minutes per sample)

• Low waste generation (<50 mL per sample)

• Cost-effectiveness

• Excellent greenness metrics across multiple evaluation tools

Clinical Significance and Practical Application

The simultaneous determination of DAP, SIT, and MET in commercial tablets is of considerable clinical importance, as this triple combination has emerged as an effective therapeutic regimen for type 2 diabetes management in patients with suboptimal glycemic control on monotherapy or dual therapy[2]. The ability to rapidly and reliably quantify all three active pharmaceutical ingredients in single analytical runs facilitates:

• Timely quality assurance and release testing

• In-process quality control monitoring

• Stability indicating method applications

• Counterfeit product detection

• Bioequivalence study support

• Routine pharmaceutical analysis

The eco-friendly nature of the method makes it particularly valuable for pharmaceutical laboratories aiming to reduce environmental impact while maintaining analytical performance, aligning with regulatory trends toward sustainability and green chemistry adoption.

CONCLUSION

A simple, cost-effective, and environmentally benign UV spectrophotometric method has been successfully developed and validated for the simultaneous determination of Dapagliflozin, Sitagliptin, and Metformin in commercial tablet formulations. The method employs the simultaneous equation approach with methanol as a common solvent, eliminating the need for complex sample preparation or specialized equipment.

Comprehensive validation according to ICH Q2(R1) guidelines demonstrated excellent specificity, linearity ($r^2 > 0.998$, Figure 7), accuracy (recovery 98-102%, Figure 8), precision (%RSD < 2%, Figure 9), and robustness (Figure 10) across critical parameter variations. Sensitivity analysis revealed adequate LOD and LOQ values for pharmaceutical quality control applications.

Greenness assessment using four independent evaluation tools (NEMI, Analytical Eco-Scale, GAPI, and AGREE) consistently demonstrated that the method achieves high environmental performance standards:

• NEMI pictogram: 2 green quadrants

• Analytical Eco-Scale: 82 (excellent green rating)

• GAPI: 7 green, 5 yellow, 3 red pentagrams

• AGREE: 0.65 (strong adherence to GAC principles

The developed method represents a significant advancement in green pharmaceutical analysis, providing an efficient, economical, and sustainable analytical solution for routine quality control testing of triple antidiabetic fixed-dose combinations while minimizing environmental impact and adhering to modern principles of green analytical chemistry. Future work should focus on extending this methodology to other pharmaceutical combinations and exploring automation possibilities for enhanced laboratory throughput.

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How to cite this article:

P. Vivek Sagar*, Simultaneous UV Spectroscopic estimation of Dapagliflozin, Sitagliptin, and Metformin in Tablets with Comprehensive Greenness Assessment Using Green Analytical Chemistry Metrics J Pharma Res, 2025; 14(06): 57-64.
DOI: https://doi.org/10.5281/zenodo.17789324

Conflict of interest: The authors have declared that no conflict of interest exists.
Source of support: Nil

