RESEARCH PAPER

Development and Validation of UV Spectrophotometric Method for the Estimation of Dapagliflozin and Metformin Hydrochloride and Its Application to Bilosomal Formulations

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ABSTRACT

The study aimed to develop and validate a stability-indicating UV spectrophotometric method for the simultaneous estimation of Dapagliflozin and Metformin, and to apply it to bilosomal nanoformulations using water as an eco-friendly solvent. The influence of sonication time and scanning interval on absorbance was evaluated, and ANOVA-guided optimization was used to establish a robust analytical condition. The developed method exhibited excellent linearity over the concentration range of 2–10 µg/mL for both drugs, with correlation coefficients (R^2) greater than 0.995. Precision studies demonstrated %RSD values below 2%, and recovery values ranged between 96.8–101.9%, confirming the accuracy and reliability of the method in accordance with ICH Q2 (R2) guidelines. Forced degradation studies under acidic, alkaline, oxidative, photolytic, and thermal stress conditions revealed clear spectral discrimination between intact drugs and degradation products, thereby confirming the stability-indicating capability of the method. The validated method was successfully applied for the simultaneous quantification of Dapagliflozin and Metformin in bilosomal formulations, demonstrating its suitability for routine quality control analysis and evaluation of nanoformulated antidiabetic therapies.

Keywords: Dapagliflozin, Metformin, UV spectrophotometry, bilosomes.

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1. INTRODUCTION

Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide and is associated with severe complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy. According to the International Diabetes Federation, the global burden of diabetes continues to rise rapidly, particularly in low- and middle-income countries, creating significant healthcare and economic challenges [1]. Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, impaired insulin secretion, and persistent hyperglycemia, which contribute to long-term vascular and metabolic complications [2]. Effective glycemic control is therefore essential to reduce disease progression and improve patient outcomes.

Metformin is considered the first-line oral antidiabetic drug for the management of T2DM. It primarily acts by decreasing hepatic glucose production, improving peripheral insulin sensitivity, and enhancing glucose uptake in skeletal muscles. Metformin is widely prescribed because of its efficacy, safety profile, affordability, and low risk of hypoglycemia [3]. However, its relatively short biological half-life and gastrointestinal side effects may limit patient compliance and therapeutic effectiveness.

Dapagliflozin is a selective sodium-glucose co-transporter-2 (SGLT2) inhibitor used for the treatment of T2DM. It lowers blood glucose levels by inhibiting glucose reabsorption in the proximal renal tubules, thereby promoting urinary glucose excretion independent of insulin action [4]. In addition to glycemic control, dapagliflozin has demonstrated beneficial effects in reducing cardiovascular and renal complications associated with diabetes [5]. Nevertheless, dapagliflozin exhibits limited aqueous solubility, which may affect its bioavailability and therapeutic performance.

To enhance the therapeutic efficacy of antidiabetic drugs, nanocarrier-based drug delivery systems such as bilosomes have gained considerable attention. Bilosomes are bile salt-stabilized vesicular systems that improve drug stability, permeability, and oral bioavailability while protecting drugs from gastrointestinal degradation [6]. These nanoformulations offer advantages such as controlled drug release, enhanced intestinal absorption, and improved patient compliance.

Various analytical methods including UV-visible spectrophotometry, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and LC–MS/MS have been reported for the estimation of Dapagliflozin and Metformin either alone or in combination [7].

Among these techniques, UV spectrophotometry remains widely preferred due to its simplicity, rapidity, cost-effectiveness, and minimal solvent consumption. However, conventional UV methods may face limitations such as spectral overlap and inability to differentiate degradation products during stability studies [8].

Although several analytical methods have been reported for the simultaneous estimation of Dapagliflozin and Metformin, limited studies are available on stability-indicating UV spectrophotometric methods applicable to nanoformulations such as bilosomes. Therefore, the present study aimed to develop and validate a simple, accurate, precise, and economical stability-indicating UV spectrophotometric method for the simultaneous estimation of Dapagliflozin and Metformin and to evaluate its applicability in bilosomal nanoformulations intended for antidiabetic therapy [9].

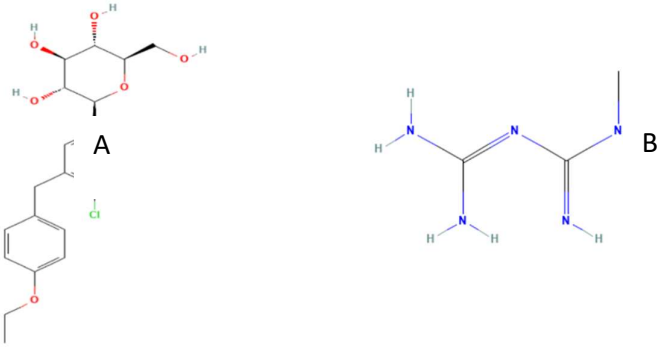

Figure 1: Chemical Structure of a) Metformin hydrochloride b) Dapagliflozin

2. Methodology

2.1. Materials

Dapagliflozin was obtained from BLD Pharma, Hyderabad, Telangana, India, while Metformin hydrochloride was procured from Bio Med Ingredients, Goa, India. All chemicals and reagents utilized throughout the study were of analytical grade and were supplied by KLE College of Pharmacy, Belagavi, India.

2.2. Instrumentation

All spectrophotometric analyses were carried out using a Shimadzu UV-1900 UV-visible spectrophotometer integrated with Lab Solutions software. Methanol was employed as the solvent blank, and all measurements were performed under ambient laboratory conditions.

2.3. Selection of Solvent

Preliminary solubility studies were conducted using various analytical solvents, including water, ethanol, methanol, and dimethyl sulfoxide (DMSO). Among the solvents evaluated, water was selected as the most appropriate solvent for method development due to its satisfactory solubility profile, spectral transparency, and eco-friendly nature.

2.4. Preparation of Stock Solution

Accurately weighed quantities of Dapagliflozin and Metformin hydrochloride (10 mg each) were separately transferred into 10 mL volumetric flasks and dissolved in methanol to prepare the primary stock solutions. Subsequently, 1 mL aliquots from each primary stock solution were further diluted to 10 mL with a methanol–water mixture (40:60 v/v) to obtain secondary stock solutions. Working standard solutions within the concentration range of 2–10 µg/mL were prepared by suitable dilution with water, followed by sonication for 5 minutes to ensure complete mixing and uniformity.

2.5. Selection of Wavelength

Standard solutions of Dapagliflozin and Metformin hydrochloride were scanned between 200–400 nm to determine their λmax values. Dapagliflozin showed maximum absorbance at 224 nm, while Metformin hydrochloride exhibited λmax at 233 nm

2.6. Method Development and Optimization

2.6.1. Establishing Analytical Target Profile (ATP) and Critical Analytical Attributes (CAAs)

The analytical method was developed using a systematic approach to obtain a simple, rapid, accurate, and cost-effective UV spectrophotometric method for the simultaneous estimation of Dapagliflozin and Metformin hydrochloride. Drug absorbance was considered the critical analytical attribute for the quantitative determination of both drugs [10,11].

2.6.2. Optimization of Analytical Conditions

Different analytical conditions such as solvent composition, sonication time, and scanning interval were evaluated to achieve optimum spectral response and reproducibility. The optimized conditions were selected based on maximum absorbance, spectral clarity, and method consistency for both Dapagliflozin and Metformin hydrochloride [12].

2.7. Method Validation

The developed UV spectrophotometric method was validated in accordance with ICH Q2 (R2) guidelines. Validation parameters including specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ),

robustness, ruggedness, and repeatability were evaluated to confirm the reliability and suitability of the method for routine analysis [13].

2.7.1. Specificity and Selectivity

Specificity of the developed method was evaluated by examining the interference caused by formulation excipients. Blank samples showed no significant absorbance at the selected wavelengths of Dapagliflozin and Metformin hydrochloride, confirming the selectivity of the method for simultaneous drug estimation.

2.7.2. Linearity

The linearity of the method was determined over the concentration range of 2–10 µg/mL by constructing calibration curves of absorbance against concentration. Regression equations and correlation coefficient (R^2) values were calculated to establish the linear relationship between concentration and absorbance.

2.7.3. Precision

Precision of the method was assessed in terms of repeatability, intraday precision, and interday precision. Three different concentrations of both analytes were analyzed in triplicate within the same day for intraday precision studies.

2.7.4. Accuracy

Accuracy of the developed method was determined by recovery studies at three concentration levels, namely 80%, 100%, and 120% of the target concentration. The percentage recovery of Dapagliflozin and Metformin hydrochloride was calculated to evaluate the closeness of the measured values to the actual drug content.

2.7.5. Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). These parameters were calculated based on signal-to-noise ratios of approximately 3:1 for LOD and 10:1 for LOQ.

2.7.6. Ruggedness

Ruggedness of the method was assessed by carrying out the analysis using different analysts and instruments under similar experimental conditions. The percentage relative standard deviation (%RSD) was calculated to verify the reproducibility of the method.

2.7.7. Robustness

Robustness was evaluated by introducing small deliberate changes in analytical parameters, such as variation in wavelength (±2 nm from λmax). The effect of these changes was assessed using %RSD values to confirm the reliability of the method under minor variations in operating conditions.

2.7.8. Repeatability

Repeatability was determined by measuring the absorbance of Dapagliflozin and Metformin hydrochloride six times at a concentration of 6 µg/mL under identical operating conditions. The %RSD values obtained were used to confirm the consistency of the method.

2.8. Forced Degradation Study

Forced degradation studies were performed to evaluate the stability-indicating capability of the developed method for Dapagliflozin and Metformin hydrochloride. The drugs were subjected to various stress conditions including acidic degradation (0.1 N HCl, 80°C, 2 h), alkaline degradation (0.1 M NaOH, 80°C, 2 h), oxidative degradation (30% H_2O_2, 80°C, 2 h), and thermal degradation (40°C, 4 h). After stress treatment, the samples were diluted to obtain a final concentration of 10 µg/mL using water and scanned over the wavelength range of 200–400 nm. Noticeable degradation under stress conditions confirmed the ability of the method to differentiate between intact drugs and degradation products [14,15].

2.9. Preparation of Bilosomes

Bilosomes were formulated using the thin film hydration technique with phosphatidylcholine (soya lecithin) and sodium deoxycholate (SDC) in a 3:1 ratio. Initially, the lipids were accurately weighed and dissolved in 10 mL of chloroform in a round bottom flask. The solvent was subsequently removed under reduced pressure using a rotary vacuum evaporator operated at 150 rpm and 60°C, resulting in the formation of a thin lipid film along the inner surface of the flask. The dried lipid film was then hydrated immediately with 10 mL of phosphate buffer saline (PBS, pH 7.4) maintained at 60°C and rotated at 150 rpm for 30 minutes to produce the bilosomal dispersion. The resulting suspension was further subjected to bath sonication for 10 minutes to achieve uniform vesicle formation and reduce particle size [19,20].

2.10. Application of the Method to Nano formulation

The validated UV spectrophotometric method was applied for the simultaneous quantification of dapagliflozin and metformin in the developed bilosomal formulation. A accurately weighed quantity of the bilosomal dispersion was taken and suitably diluted with distilled water. The sample was subjected to sonication to ensure complete disruption of bilosomal vesicles and release of the entrapped drugs. The resulting solution was filtered and further diluted appropriately to fall within the linearity range of the developed method. The absorbance of dapagliflozin and metformin was then measured at their respective λmax values using a UV–Visible spectrophotometer. The drug content was calculated using the previously established calibration curves [22,23].

2.11. Greenness Assessment of the Developed Method

The sustainability of the developed UV spectrophotometric method for simultaneous estimation of dapagliflozin and metformin was

evaluated using Complex GAPI, AGREE, and BAGI tools, confirming its minimal environmental impact and suitability for routine analysis.

Complex GAPI was applied to visually assess the environmental impact across all stages of the analytical procedure, including sample preparation, solvent usage, instrumentation, energy consumption, and waste generation. The predominance of green and light-yellow sections in the GAPI profile indicates the use of eco-friendly solvents (water), minimal sample preparation steps, low energy requirements, and reduced waste generation, thereby confirming the environmentally sustainable nature of the developed UV method [16].

The Analytical Greenness (AGREE) metric was further employed to quantitatively evaluate compliance with the twelve principles of Green Analytical Chemistry. The AGREE tool provides a normalized greenness score ranging from 0 to 1 with a circular pictographic output for easy interpretation. Application of this metric to the developed method demonstrated a high greenness score, which reflects minimal solvent consumption, reduced energy usage, and simple instrumental requirements, thereby confirming the eco-friendly profile of the UV spectrophotometric method for dapagliflozin and metformin analysis [17].

In addition, the Blue Applicability Grade Index (BAGI) was utilized to integrate green analytical chemistry principles with practical applicability parameters. BAGI considers environmental impact as well as analytical performance, operational simplicity, cost-effectiveness, energy demand, and analyst safety. The high BAGI score obtained for the proposed method indicates strong methodological whiteness, reflecting an optimal balance between sustainability and analytical robustness. Overall, the greenness assessment confirms that the developed UV spectrophotometric method is environmentally benign, analytically reliable, and suitable for routine quantification of dapagliflozin and metformin in bilosomal drug delivery systems [18].

3. Results and Discussion

3.1. Selection of Solvent and Wavelength

Water was selected as the optimal solvent based on its excellent spectral clarity, low toxicity, and adequate solubility for both dapagliflozin and metformin. All experimental analyses were carried out using water as the blank. The standard solutions of both drugs (10 µg/mL) were scanned in the UV range of 200–400 nm. Dapagliflozin exhibited maximum absorbance at 224 nm, while metformin showed λmax at 233 nm using methanol as the solvent.

3.2. Method Development and Optimization

The UV spectrophotometric method was developed for the simultaneous estimation of dapagliflozin and metformin in bilosomal formulations. The method was optimized by selecting appropriate detection wavelengths that provided adequate sensitivity and minimal interference between the two drugs. The selected λmax values ensured good resolution and reliable quantification of both analytes. The optimized method demonstrated satisfactory linearity, accuracy, and precision, making it suitable for routine analysis of the developed bilosomal drug delivery system.

3.3. Method Validation

Method validation is a critical step to ensure regulatory compliance, adherence to scientific standards, and fulfillment of quality control requirements. The developed method was systematically validated in accordance with ICH Q2 (R1) guidelines.

3.3.1. Specificity and Selectivity

The developed UV spectrophotometric method showed good specificity and selectivity for the simultaneous estimation of dapagliflozin and metformin in bilosomal formulation. Dapagliflozin and metformin exhibited distinct absorption maxima at 224 nm and 233 nm, respectively, allowing their accurate identification and quantification without overlap.

The blank (methanol) showed no absorbance at the selected wavelengths, confirming no interference from solvent or excipients and ensuring reliable and selective estimation of both drugs in bilosomal formulation for routine analysis.

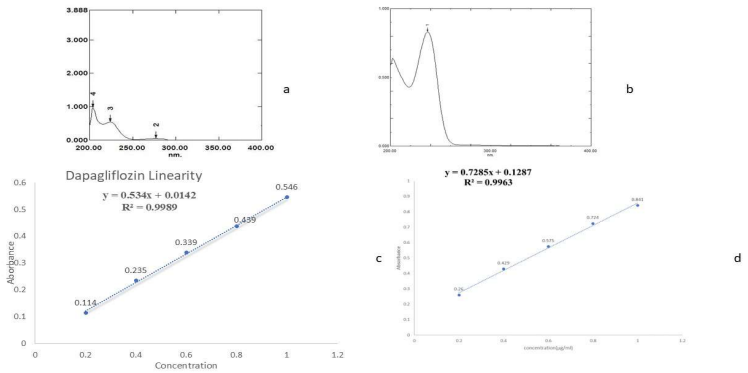

Figure 2. A: UV spectrum of Dapagliflozin, B: UV spectrum of Metformin C: Calibration curve of Dapagliflozin, D: Calibration curve of Metformin

3.3.2. Linearity

Linearity of the method refers to its ability to produce results that are directly proportional to the concentration of analyte within a given range. The strong correlation between concentration and absorbance was confirmed by a linear relationship observed in the concentration range of 2–10 µg/mL for both dapagliflozin and metformin. The regression coefficients were found to be 0.9963 for dapagliflozin and 0.9989 for metformin, indicating excellent linearity of the method.

Sr. no.	Concentration (µg/ mL)	Absorbance of Dapagliflozin (224 nm)	Absorbance of Metformin (233 nm)
1	0	0.000	0.000
2	2	0.168	0.175
3	4	0.329	0.175
4	6	0.488	0.501
5	8	0.651	0.668
6	10	0.812	0.824
R^2		0.996	0.998
LOD		0.262 µg/mL	0.310 µg/mL
LOQ		0.93 µg/mL	0.40 µg/mL

Table 1. Linearity of Dapagliflozin and Metformin.

3.3.4. System Precision

The precision of the developed method was evaluated through intraday and interday studies. Intraday precision was determined by measuring absorbance at three different time intervals within the same day (morning, afternoon, and evening), while interday precision was assessed by repeating the analysis over three consecutive days. The method demonstrated good precision, as all %RSD values were found to be less than 2%. The detailed results are presented in Tables 2and 3.

Table 2. Intraday precision assay

Table 3. Interday precision assay

Dapagliflozin Intraday precision (n=3)				
Concentration (µg/ml)	%RSD	Morning	Afternoon	Evening
2	1.37	0.187	0.184	0.182
6	0.34	0.510	0.510	0.507
10	0.12	0.833	0.832	0.831
Metformin Intraday precision (n=3)				
Concentration (µg/ml)	%RSD	Morning	Afternoon	Evening
2	1.64	0.179	0.174	0.174
6	0.115	0.503	0.504	0.503
10	0.185	0.825	0.822	0.824
Dapagliflozin Interday precision (n=3)				

Concentration (µg/ml)	%RSD	Day 1	Day 2	Day 3
2	1.35	0.189	0.187	0.184
6	0.496	0.510	0.507	0.505
10	0.368	0.833	0.829	0.827
Metformin Interday precision (n=3)				
Concentration (µg/ml)	%RSD	Day 1	Day 2	Day 3
2	1.17	0.179	0.178	0.175
6	0.61	0.503	0.499	0.497
10	0.36	0.825	0.822	0.819

3.3.5Accuracy

Accuracy describes the closeness of agreement between the true value and the measured value obtained by the developed method. The accuracy of the proposed UV spectrophotometric method was evaluated by recovery studies at different concentration levels.

The percentage recovery for dapagliflozin ranged from 96.8% to 101.6%, while that of metformin ranged from 97.7% to 101.9%. The consistent and reproducible recovery values across all levels indicate that the method is accurate and reliable for routine analysis. The detailed results are presented in Table 4.

Table 4. Accuracy/ percentage recovery

LEVEL	Concentration (µg/ml)	Absorbance (224 nm)	% Recovery	Absorbance (232 nm)	% Recovery
80%	4	0.341	101.6%	0.334	101.9%
		0.338		0.331	
		0.340		0.333	
100%	6	0.510	99.4%	0.503	101.0%
		0.490		0.489	
		0.489		0.482	
120%	8	0.647	96.8%	0.630	97.7%
		0.639		0.631	
		0.641		0.636	

3.3.6. Sensitivity

The sensitivity of the developed method was assessed by determining the limits of detection (LOD) and quantification (LOQ). The LOD was calculated using 3.3 times the ratio of the standard deviation of the intercept to the slope of the calibration curve, while the LOQ was calculated using 10 times the same ratio.

For the developed method, the LOD and LOQ were found to be 0.248 µg/mL and 0.751 µg/mL for dapagliflozin, and 0.304 µg/mL and 0.921 µg/mL for metformin, respectively. These results indicate that the method is highly sensitive and suitable for accurate trace-level quantification of both drugs.

3.3.7. Robustness and Ruggedness

Robustness refers to the ability of an analytical method to remain unaffected by small, deliberate variations in method parameters, indicating its reliability under normal laboratory conditions. In this study, robustness was evaluated by slightly varying the detection wavelengths (224 ± 2 nm for dapagliflozin and 233 ± 2 nm for metformin) and observing the corresponding changes in absorbance.

Sr. No.	Concentration (µg/ml)	Absorbance Dapagliflozin	% RSD	Absorbance Metformin	% RSD
1	6	0.510	1.62	0.503	0.77
2	6	0.493		0.483	
3	6	0.490		0.489	
4	6	0.497		0.491	
5	6	0.502		0.498	
6	6	0.489		0.482	

Ruggedness represents the reproducibility of results under different operational conditions. It was assessed by performing the analysis of the same samples at concentrations of 2, 6, and 10 µg/mL by two different analysts. The method demonstrated good ruggedness, as the %RSD values for both robustness and ruggedness studies were found to be less than 2%, confirming the method's consistency and reliability. The detailed results are presented in Tables 5 and 6.

Table 5. Ruggedness with change in analyst

Dapagliflozin				Metformin	
Concentration (µg/ml)		Change in Analyst			
		Analyst 1	Analyst 2	Analyst 1	Analyst 2
2	%RSD	0.571	1.28	1.15	0.897
6		0.507	0.717	0.416	0.736
10		0.187	0.509	0.329	0.178

Table 6. Robustness with change in wavelength

Dapagliflozin				Metformin	
Concentration (µg/ml)		Change in Wavelength			
		237 nm	241 nm	229 nm	233 nm
2	%RSD	0.998	1.01	0.996	1.35
6		0.781	0.276	0.573	0.16
10		0.342	0.353	0.268	0.358

3.3.8. Repeatability

Repeatability, also referred to as intra-assay precision, indicates the consistency of results obtained under identical operating conditions over a short time period. A %RSD value of less than 2% reflects excellent repeatability of the method. The results are summarized in Table 7.

Table 7: Repeatability

3.3.9. Forced Degradation Study

Forced degradation studies were carried out to evaluate the stability of the drugs under acidic, alkaline, oxidative, and photolytic conditions. Significant degradation was observed under oxidative and alkaline environments, indicating higher susceptibility under these conditions. The results confirm that the developed method is stability-indicating in nature (Table 8).

Forced Degradation Assay	Degradation	
	Dapagliflozin	Metformin
Acidic	12.94%	14.23%
Basic	26.12%	100%
Oxidative	100%	100%
Sunlight	25.67%	26.22%

Table 8: Results of forced degradation study

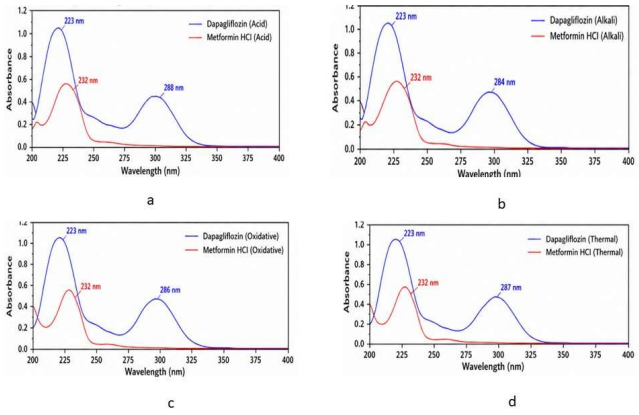

Figure. 3. Forced Degradation assay of dapagliflozin and Metformin

3.3.10. Estimation of Dapagliflozin and Metformin in Bilosomes

With the growing development of nanocarrier-based drug delivery systems, reliable and precise analytical methods are essential for proper quality evaluation. Accurate quantification of drugs encapsulated within bilosomal formulations is crucial, as it directly influences therapeutic effectiveness and formulation performance. In this study, the validated UV spectrophotometric method was successfully applied for the estimation of dapagliflozin and metformin in bilosomal formulations.

Table 9. Estimation of Dapagliflozin and Metformin from nanoformulation

	Particle size	% EE	Zeta potential
BILOSOMES	242.7 nm	92.02	-42.16mV

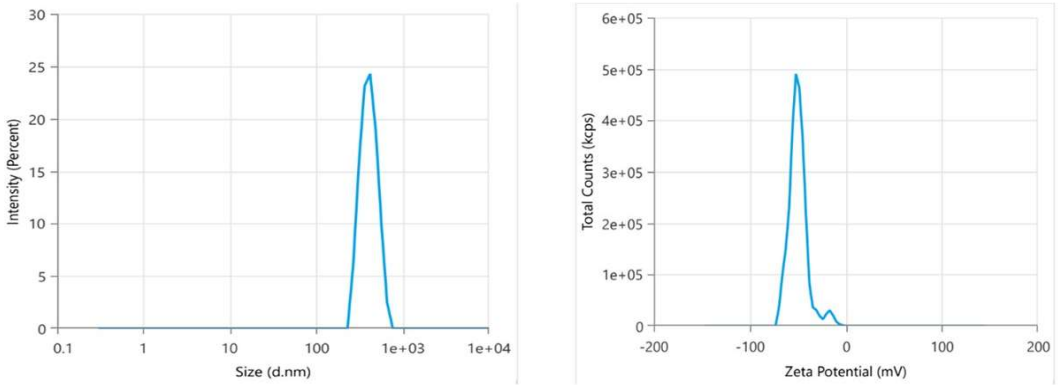

Figure 4. Particle size (a) & Zeta Potential (b) of the Optimized formulation

3.3.11. Assessment of Method Greenness and Whiteness Attributes

The environmental sustainability of the developed UV spectrophotometric method for dapagliflozin and metformin was evaluated using the Complementary Green Analytical Procedure Index (ComplexGAPI). The pictogram (Fig. 8a) displayed seven green, nine yellow, and one red segment, indicating an environmentally acceptable method. The green sections correspond to the use of low-toxicity solvents and minimal solvent consumption, while the yellow segments are mainly associated with sample preparation steps and instrument energy requirements. The central E-factor value of 1.0 further confirms low waste generation, reflecting good compliance with green analytical chemistry principles.

The greenness profile was further assessed using the Analytical GREEnness (AGREE) metric, which quantitatively evaluates adherence to the twelve principles of green analytical chemistry. The method achieved a high AGREE score of 0.8, and the corresponding pictogram showed a predominantly green profile, confirming its strong environmental compatibility and minimal ecological impact (Fig. 5).

In addition, method whiteness was evaluated using the Blue Applicability Grade Index (BAGI), which integrates analytical performance, environmental impact, and practical applicability into a single parameter. The obtained BAGI score of 70 indicates a well-balanced analytical method with good robustness, efficiency, and environmental safety, supporting its suitability for routine analysis of dapagliflozin and metformin.

Overall, the greenness and whiteness assessments confirm that the developed UV spectrophotometric method achieves an effective balance between analytical reliability, environmental sustainability, and operational efficiency, making it suitable for routine pharmaceutical quality control applications.

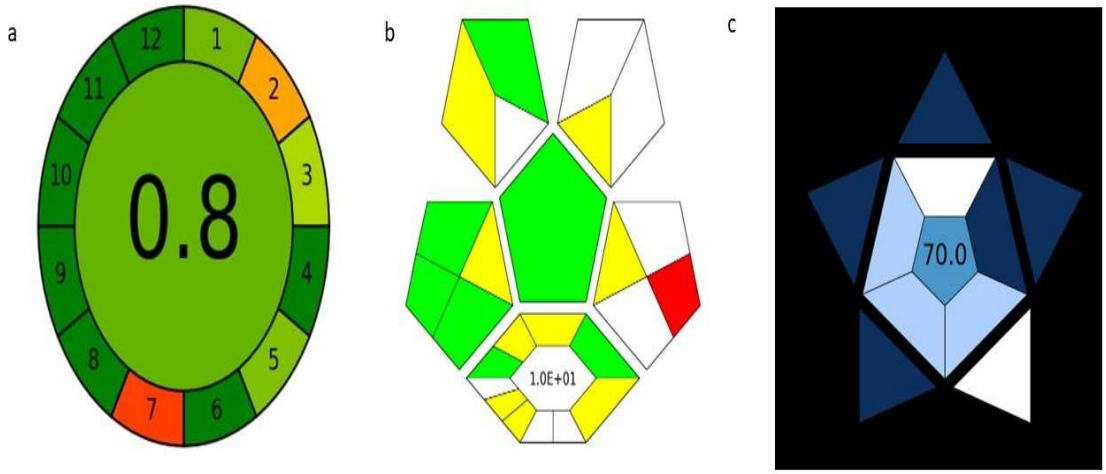

Figure 5. a) GAPI pictogram representing the greenness profile of the developed UV spectrophotometric method, b) AGREE pictogram representing the green analytical profile of the developed UV spectrophotometric method, c) BAGI pictogram representing the greenness profile of the developed UV spectrophotometric method.

4. Conclusions

A simple, sensitive, and stability-indicating UV spectrophotometric method was successfully developed, optimized, and validated for the simultaneous estimation of dapagliflozin and metformin. The method demonstrated good linearity, accuracy, precision, and robustness in accordance with ICH Q2(R1) guidelines, confirming its reliability for routine analysis. Forced degradation studies confirmed that the method can effectively distinguish the drugs from their degradation products, indicating its stability-indicating nature.

The method was successfully applied to bilosomal nanoformulations, demonstrating its suitability for complex drug delivery systems. Overall, the developed method is cost-effective, rapid, and environmentally friendly, making it a valuable analytical tool for routine quality control and pharmaceutical research applications.

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Author contributions

Author 1- Conceptualization, Investigation, Methodology, Software, Writing- Original draft, Author 2- Project administration, Supervision, Writing- Review & editing.

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Disclosure of conflicts

The authors state that there are no conflicts of interest or financial associations that may have affected the results or conclusions of this study.

Use of artificial intelligence (AI)-assisted technology

The authors confirm that no artificial intelligence (AI) tools were used in the writing or editing of the manuscript, and that no images were created or modified using AI.

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