Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 335-353.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

IMPLEMENTATION OF VALID UV-SPECTROPHOTOMETRIC METHOD FOR QUANTIFICATION OF DACOMITINIB IN PURE FORM AND NANO-DRUG DELIVERY SYSTEMS: A GREENNESS ASSESSMENT USING ESTABLISHED METRICES

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Dacomitinib (DMB), is a chemotherapeutic drug recently approved by FDA rendering it as fresh candidate that is expected to be heavily subjected to research in the near future especially in the nanotechnology field. The study aimed to develop a valid economic process for quantifying DMB in both pure and pharmaceutical dosage forms using UV-visible spectroscopy method. Cubosomes are interesting nanocarriers that have shown a great role in successful delivery of chemotherapeutic drugs to different tumor sites, so they were selected for DMB encapsulation to increase our challenge. Measurement of wavelength maximum for DMB in different solvents was conducted followed by construction of absorbance curves to detect regression values. For further justification our developed method was statistically compared to one of the rare methods found for determination of DMB which was HPLC determination for the drug in its pure form or encapsulated in cubosomes. Validation was accomplished conferring to the international conference on harmonization (ICH) guidelines. The produced data indicated that the developed approach was an economic analysis method, precise and complies with the recommendations of ICH guidelines that could be used in the future determination of drug as evidenced by the fact that the RSD was less than 2% , good linearity was achieved in the range of 2-14 ug/mL and the LOD and LOQ was 0.47 and 1.4 µg/mL respectively. The greenness of the method was assessed using GAPI and AGREE metrices, indicating its potential for environmentally sustainable application.

***Keywords**: Dacomitinib; UV-Visible Spectrophotometer; Cubosomes; HPLC; Greeness*

INTRODUCTION

Cancer is a fatal medical condition that threatens 180 countries worldwide, with 19.1 million new cancer cases reported in 2020 According to WHO. Lung cancer is noted as the most dominant lethal type of cancer globally[1] . Small molecules Tyrosine Kinase Inhibitors (TKIs) are considered as the most prominent first line chemotherapies for the treatment of lung carcinoma, especially non-small cell lung cancer (NSCLC) which contain specific genetic mutations in the Epidermal Growth Factor Receptors (EGFR)[2]. Tyrosine kinase inhibitors (TKIs), which are nanomaterials designed to inhibit cell signaling by blocking signal transduction pathways, have brought new hope to cancer treatment in recent times. However, formulation scientists must address several formulation challenges associated with TKIs to enhance the effectiveness of current drugs[3]. Cancer nanotherapeutics, utilizing nanocarriers for drug delivery, have emerged as an advanced approach to tackle these formulation issues and improve cancer treatment. This strategy offers superior active and passive drug targeting, overcoming the limitations of conventional

Received : 9/11/2024 & Accepted : 9/3/2025

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cancer therapies.[4] First line TKIs (Erlotinib and Gefitinib) have shown efficient therapeutic responses at the beginning of their use, yet recurrence due to resistance to drugs occurred in about 60% of EGFR lung carcinoma cases[3,5]. Hence development a second-generation of TKIs as Dacomitinib (DMB) was investigated to overcome the acquired resistance observed with first-line EGFR TKIs[6].

The Food and Drug Administration (FDA) accepted DMB in the form of VIZIMPRO® tablets in where it has shown promising results for metastatic lung cancer patients accompanied with significant survival rates[7]. The scientific name of DMB is (2E)-N-{4-[(3-Chloro-4fluorophenyl) amino] -7-methoxyquinazolin-6-yl}-4-(piperidin-1-yl) but-2-enamide and its chemical structure is illustrated in **Fig. 1** with 487.95 Daltons molecular weight. Physically, DMB is considered a dry water- insoluble buff powder with slight solubility in some organic solvents such as acetic acid, acetone, and acetonitrile. From Biopharmaceutical side, it illustrates low *in-vivo* dissolution accompanied with high permeability (BCS Class II). Dacomitinib is currently available only in tablet form. When administered orally, it can cause severe diarrhea, necessitating dose modifications. Therefore, further research is needed to explore alternative routes of administration.[8]

To our best knowledge, only one HPLC analytical method[9], one RP-HPLC[10]**, two** LC-MS methods[6,11] and one UPLC method[12] were reported for the analysis of DMB in solution, DMB impurities. solid dosage form, biological samples and pharmacokinetic studies respectively. Recently Flourescence Quantum dots approach was developed to determine DMB concentration in bulk solutions[13]. Hence, DMB is a fresh candidate that is expected to be heavily subjected to research in the near future especially in nanomedical studies,[14]. The current study aimed to implement a valid simple, rapid, reliable, precise and economic spectrophotometric method for the analysis of DMB in both solution and nano drug delivery system. Cubosomes are interesting nanocarriers that have shown a great role in successful delivery of chemotherapeutic drugs to different tumor sites, so they were selected for DMB encapsulation to increase our challenge[15]. Cubosomes show unique characterization such as fine particular size, large surface area and high encapsulation capacity[16]. As shown later, the proposed method yielded satisfactory accuracy and precision. This makes the developed method suitable for routine DMB analysis in quality control and research as UV spectrophotometric analysis is widely used in Pharmaceuticals, chemistry and environmental studies for its accuracy and efficiency[17,18,19] Hence, the need for a novel DCB determination method that is straightforward, cost-effective, and environmentally friendly prompted the authors to undertake this study. The novelty of our study lies in the introduction of a new drug and its unique formulation. This distinction offers fresh insights and potential therapeutic implications that have not been previously explored. For further justification our developed method was statistically compared to HPLC determination.

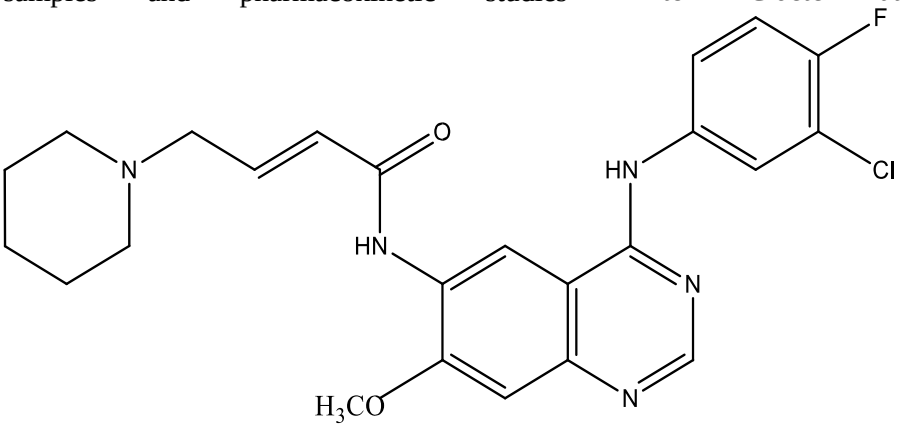

Fig 1: Chemical Structure of Dacomitinib.

Methodology

MATERIAL AND METHODS

Dacomitinib (m wt: 488.0, purity ≥ 99%) and Poloxamer (P 407, purity ≥ 98%) were Procured from Sigma Aldrich (Steinheim, Germany)**.** Glyceryl monooleate (GMO, purity ≥ 98%), was generously given for research purposes from Gattefosse (Lyon, France). Ethanol and Acetonitrile (HPLC grade, purity ≥ 99.9%) and all other chemicals of analytical grade (purity ≥ 99%) were obtained from Chemajet (Cairo, Egypt)**.** Phosphate Buffer was freshly made in accordance with EP.

Scanning of Dacomitinib in different solvents

A specified amount of DMB was dissolved in various solvents viz; Ethanol, 0.1N HCL, phosphate buffer Saline (pH 5.6), and phosphate buffer Saline (pH 7.4). The maximum absorbance (λ_{max}) of DMB in different prepared media were scanned spectrophotometrically (A JASCO V-530 double beam, Japan).

Construction of calibration curve of Dacomitinib in different dissolution media

Standard Solution obtained by dissolving precisely weighed, 10 mg of DMB in Ethanol, 0.1N HCL, phosphate buffer Saline (pH 5.6), and phosphate buffer Saline (pH 7.4) using a stoppered measuring flask (100 mL capacity). The concentration of the prepared solution was 100µg/mL. From this stock solution different volumes were withdrawn and completed to 10 mL to give concentrations of 2, 4, 6, 8, 10, 12, 14 µg/mL. The Ultraviolet absorbance of the different serial concentrations was measured at the preset λ_{max} against a blank. A calibration curve and regression line were drawn by putting the measured absorbances against the corresponding DMB concentrations. The equation of the curve was estimated among different dissolution media.

Validation procedures

The implemented method was subjected to different validation procedures that were extracted from the ICH Guidelines for the following parameters which are crucial for ensuring reliable and valid results **(ICH Guideline, Q2 (R1), 2005)**:[20,21]

Linearity

Linearity refers to the ability of the analytical method to elicit test results that are directly proportional to the concentration of analyte in the sample within a given range. It ensures that the method can accurately reflect changes in concentration. Aliquots with concentrations ranging from 1 to 20 ug/mL were assayed in triplicate. The outcomes attained were used to compute linear regression using the least squares regression method.

Precision

Precision refers to the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. It includes repeatability and reproducibility. The Repeatability and Intermediate precision of the proposed spectrophotometric determination technique were detected over a one-week period by analyzing the corresponding response triplicate for one day and along three other various days, and the findings were presented as percentage relative standard deviation values.

Accuracy

The method's accuracy is the nearness of the obtained value compared to the practical value for the sample. It indicates how close the measured value is to the actual value. For the study, three different concentrations of the tested DMB solution were set, and the absorbance of each solution was measured in triplicate.

Sensitivity of method

It is the least concentration of drug in a tested sample that can be detected without being quantified that can be determined by the following equation,

LOD = 3.3 X (standard deviation of the intercept / slope of the calibration curve)

Whereas the lowest amount of drug that can be quantified at an accepted level of accuracy and precision.

LOQ = 10 X (standard deviation of the intercept / slope of the calibration curve)

Robustness

The robustness of the proposed method is a measure of the analytical method's capacity to stay unaffected by tiny but deliberate variations in the method parameters such as changing the wavelength range or the slit width.

These parameters collectively ensure that the UV spectrophotometric analysis of a drug is reliable, accurate, and consistent, thereby providing confidence in the results and ensuring the drug's quality and efficacy.[21,22]

Preparation of Dacomitinib loaded Cubosomes

DMB-loaded cubosomal dispersion was prepared and framed by the Top down approach basing to the technique published by[23,24]. The lipid phase components and the stabilizer (500 mg GMO and 50 mg P 407) were heated at 60 °C on a magnetic stirrer (MSH-20D, Korea) until they were homogenous, and 10 mg of DMB was dispersed in the molten lipids under continuous stirring. Certain volume of deionized water (0.5 mL) heated to the same temperature and dropped to the mixture at 2000 rpm stirring speed to achieve homogeneity. The mixture was allowed to equilibrate for 48 hours at room temperature. The resulting gel was magnetically stirred with another amount of deionized water (19.5 mL) for 5 minutes before being sonicated for 4 minutes using the Probe Sonicator. (UP50H/ UP100H Hielscher Ultrasonics GmbH).

Characterization of cubosomal dispersion
Entrapment Efficiency (EE %)

The percentage of DMB entrapped in each formulation was determined in triplicate. First, the total amount of DMB incorporated in 1 mL of cubosomal dispersion was measured by adding 9 mL of ethanol to the dispersion and analyzing the resulting solution for its total DMB content. Then, the amount of free (unentrapped) DMB was assessed using an ultrafiltration centrifugation technique. Specifically, 1 mL of DMB-loaded cubosomal dispersion was diluted with deionized water to a final volume of 10 mL. Then, 3 mL of the diluted sample was filtered through Amicon Ultra 3000 MWCO centrifuge tubes (Millipore, USA) and centrifuged at 5000 rpm for 15 minutes. The free DMB present in the filtrate was quantified. Entrapment efficiency (EE%) was then calculated using the following Equation:

$$EE\% = \frac{\text{Total amount of drug in 1 mL} - \text{Amount of free drug in filtrate}}{\text{Total amount of drug in 1 mL}} \times 100$$

Particle size and Zeta Potential

The particle size (Z-average), zeta potential, and polydispersity index (PDI) of the cubosomal nanoparticles were analyzed using dynamic light scattering with a Malvern Zetasizer (Malvern Instruments, Worcestershire, UK). For each formulation, a 1 mL sample was taken and diluted with 29 mL of deionized water. The measurements were performed in triplicate at a temperature of 25 ± 0.5 °C.

Transmission Electron Microscope (TEM)

To examine the morphology of cubosomal nanoparticles, a transmission electron microscope (JEOL, Japan, model JEM-2100) equipped with a super twin lens was utilized. A droplet of the cubosomal dispersion was placed onto a carbon-coated copper grid and stained with a 1% sodium phosphotungstate solution. Excess fluid was then removed using absorbent filter paper, and the sample was allowed to dry at room temperature for 15 minutes before imaging.

Selectivity evaluation

To evaluate the selectivity of the developed spectrophotometric method, UV spectra of placebo cubosomes (formulations without Dacomitinib) and commonly used excipients were recorded under identical conditions as the drug-containing samples. Placebo cubosomes were consistently used as blanks during absorbance measurements to minimize potential interference. Additionally, the potential impact of biological fluids was assessed using plasma samples spiked with Dacomitinib. These samples underwent appropriate preparation before spectral analysis to ensure accurate evaluation of interference.

HPLC detection of DMB in solution and cubosomes

The amount of DMB either in solution or incorporated into cubosomes was detected by HPLC for the sake of confirmation and validity of the developed spectrophotometry method. An HPLC method for determination of the drug in both formulation and dissolution media was described by[9]. The HPLC analysis of DMB was performed using HPLC detector coupled to Waters UV/visible detector. The column used was C18 column at 35°C (5 µm, 4.6 × 250 mm, Waters Corporation, Milford, MA, USA). An isocratic elution of mobile phase consisting of a blend of buffer and acetonitrile with the ratio of (75:25) used to elute the stationary phase (silica bonded with octadecylsilane) at elution rate 1 mL/min. The buffer was prepared as 5 mL triethylamine in 1000 mL de-ionized water and pH 3.2 ± 0.1 adjusted with orthophosphoric acid. All the solutions were filtered through a 0.22 membrane filter and degassed by sonication. The data was reported using Empower 2 software and the detector was set at maximum wavelength of DMB. In a volumetric flask, five mg of DMB were dissolved in 20 mL ethanol, and volume was made up to 100 m l with the mobile phase. A group of serial dilutions were performed in concentrations ranging from 0.5 to 20 µg/mL. A calibration curve was constructed, accuracy, and recovery studies were carried out. A weighted 10 mg of Cubosomes were dispersed in the mobile phase and then injected into HPLC equipment to determine the amount of DMB encapsulated in Cubosomes.

Statistical investigation

All the results were analyzed using the Graph pad prism program using the paired samples T test where $P < 0.05$ was directed as the level of significance.

Evaluation of the Greenness of the Procedures

The greenness of the analytical procedures was evaluated using two specific metric tools. A brief overview of how each tool assesses environmental performance is provided in the following sections.

Green Analytical procedure Index (GAPI)

GAPI offers significant benefits for evaluating analytical methods by accounting for the environmental impact of materials used prior to the analysis itself[25] This assessment tool systematically evaluates each step of the analytical procedure across four main categories with a total of 15 parameters, which encompass aspects such as sample collection, transportation, preservation, preparation, the reagents and compounds involved, as well as the instrumentation and intended purpose of the method. The results of the GAPI assessment are visually represented through five pentagrams, with a color scheme where green indicates minimal environmental impact, yellow signifies moderate impact, and red denotes a high environmental impact.

Analytical Greenness (AGREE)

The Analytical Greenness (AGREE) metric tool is a recent addition for evaluating the environmental sustainability of analytical procedures. AGREE is a user-friendly software that generates clear, easily interpreted results. The greenness assessment criteria in AGREE are based on the 12 principles of Green Analytical Chemistry (GAC), encapsulated in the word "SIGNIFICANCE" and described in detail in the foundational article by[26]. Results are displayed in a circular pictogram divided into 12 sections around the edge, with each section color-coded from deep green (=1) to deep red (=0) to represent its ecological impact. An overall score, calculated as a fraction of one, is displayed in the center of the pictogram.

RESULTS AND DISCUSSION

Scanning of Dacomitinib in different solvents

Research on dacomitinib is ongoing, with numerous studies continuing to explore its pharmacological properties, efficacy, and broader clinical applications in oncology[27,28] . Actually The study aimed to design a pH-sensitive cubosomes formulation for the selective and efficient targeted delivery of Dacomitinib to enhance its accumulation at the tumor site while minimizing systemic exposure and off-target effects as Tumor extracellular environments are more acidic, with pH levels between 6.5 and 6.9, while endosomes and

lysosomes exhibit even lower pH values, ranging from 5.0 to 5.5. Also pH 7.4 to mimic blood pH. Results of spectrophotometric scanning of DMB in Ethanol, 0.1N HCL, Phosphate Buffer Saline (PH 5.6) and (pH 7.4) were presented in **Fig. 2 a, b, c and d**. Though the polarity and pH of solvents showed high variation, the scanning process showed that there were no differences in the λ_{max} of DMB in the Four solvents and it was recorded as 254 nm[14,29] .The difference in wavelengths (254 nm in this study versus 259 nm in [29]) is unlikely to be significant. Variations in results may instead stem from other factors, such as differences in solvent composition or experimental condition. The result confirmed the stability and the accuracy of the determined λ_{max}. These solvents were carefully selected to represent different media investigated in the advanced research of nanotechnology.[30]

Linearity

A linear response ensures that calibration curves are accurate and that the drug concentration can be reliably determined from the spectrophotometric readings. The linearity studies were carried out by graphing the concentrations of the measured standard mixture solution against the respective absorbance as shown in **Fig. 3** . **Table 1** illustrates 4 Different dissolution media containing DMB showed linearity within the concentration between 2-14 µg/mL. The correlation coefficient values were approximately 0.999, and the calibration curve demonstrated that DMB obeyed Beer's law limit in the studied concentration range.

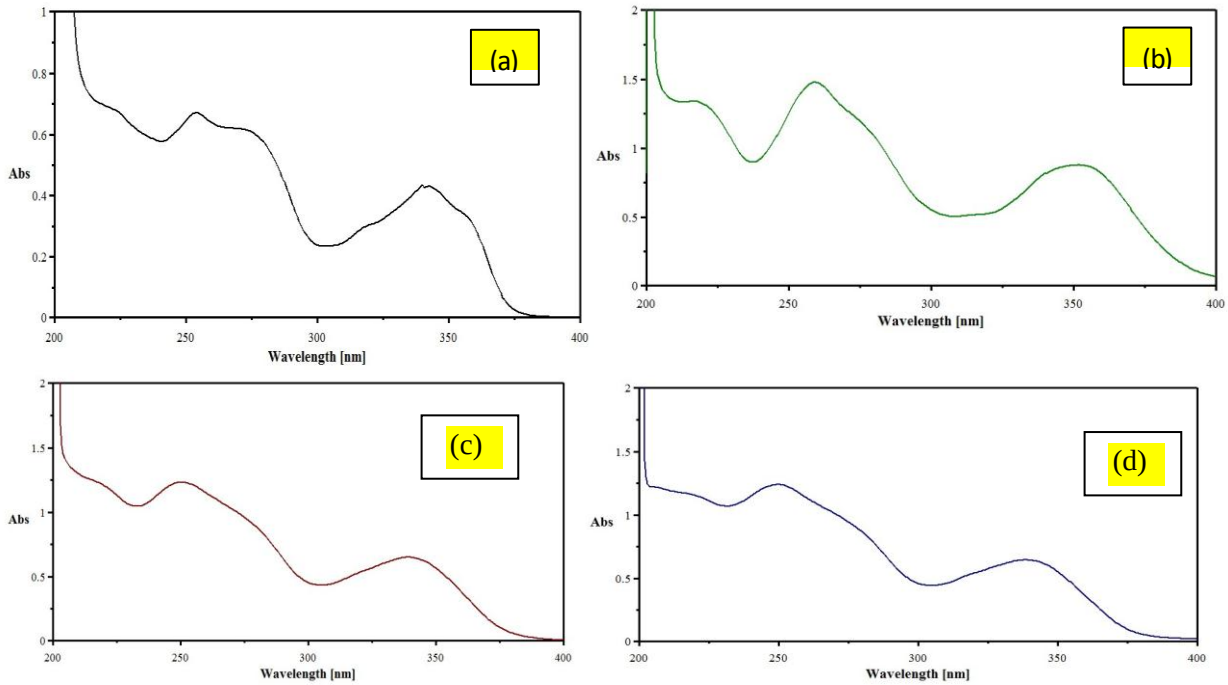

Fig 2: The scanning of Dacomitinib in Four different solvents: (A) Ethanol, (B) 0.1N HCL, (C) PBSPH 7.4, (D) PBS PH 5.6.

Table 1: Regression parameters of DMB in different dissolution media.

Solvent	Beer's Law limit ug/mL	λmax (nm)	Linear Regression Equation	R^2	Molar Absorptivity Value
Ethanol	2 -14	254	A=0.0674x-0.0056	0.999	0.067
0.1N HCL	2 -14	255	A = 0.073x - 0.0349	0.998	0.075
PBS (pH 5.6)	2 -14	253	A = 0.0673x - 0.0246	0.999	0.067
PBS (pH 7.4)	2 -14	254	A = 0.065x + 0.0312	0.998	0.065

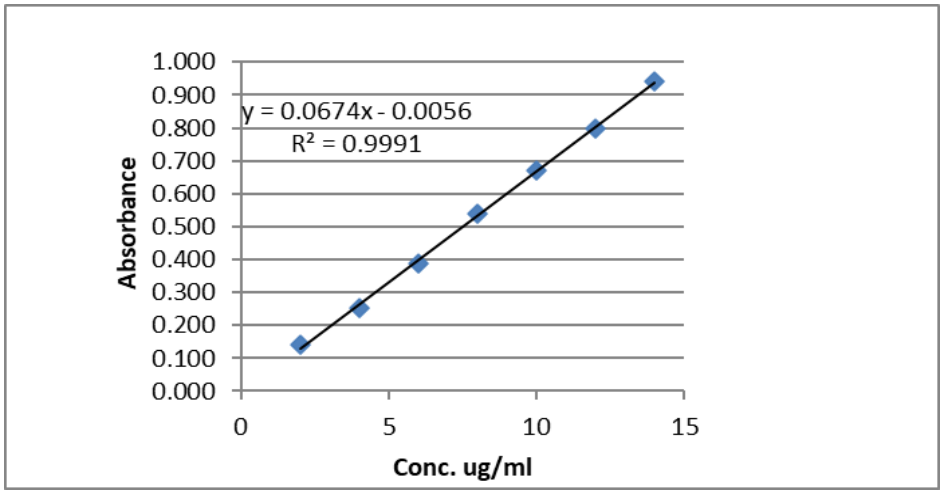

Fig 3: Calibration curve of Dacomitinib in Ethanol at λ_{max} 254 nm.

The values of slopes were recorded as 0.0674, 0.073, 0.0673 and 0.0648 with Ethanol, 0.1N HCL, PBS (pH 5.6), PBS (pH 7.4) respectively. Furthermore, **Fig. 4** noted the overlay spectra of DMB supporting the results of linearity. The Percent error values were not more than 2%, which indicates the validity of the developed proposed method. Based on these results subsequent investigations will be held on DMB ethanolic solution as a model for other solutions. All the statistical parameters are demonstrated in **Table 2**.

In comparison with the previous literature, the linearity range obtained in this study shows close agreement with those reported for sunitinb malate, is a member of the tyrosine kinase inhibitor family[31], which demonstrated a linear range of 2-12 µg/mL with an r^2 value of 0.995. However, other studies, such as microwell spectrophotometric assay[29], observed a slightly different range (4-90 µg/mL), which may be attributed to variations in analytical conditions such as solvent composition, wavelength settings, or sample preparation techniques. These differences highlight the robustness of our method across a concentration range similar to or slightly narrower than those reported in the literature.

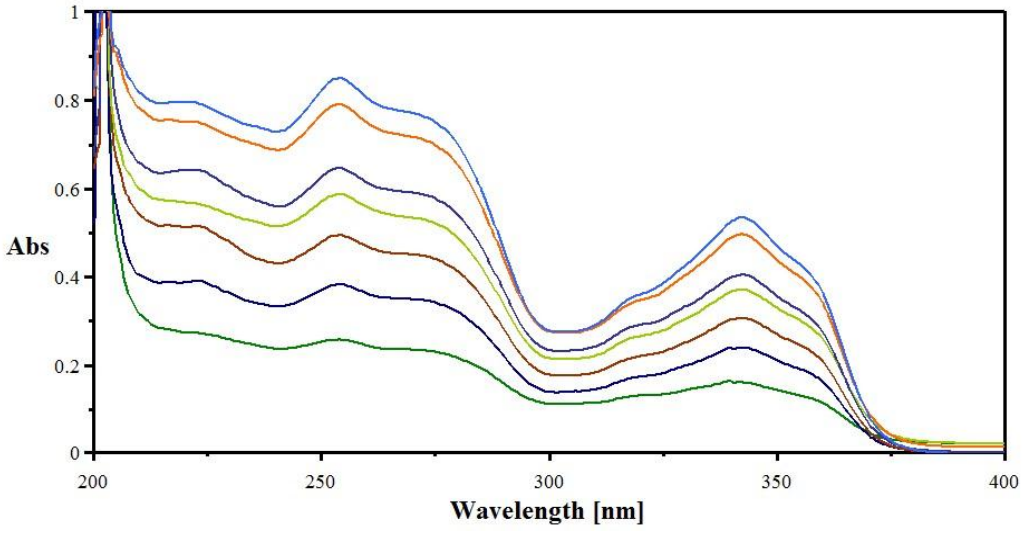

Fig 4: Overlay absorption spectrum of DMB in ethanol in the conc. Range 2-14 ug/mL

Table 2: Statistical Validation Parameters of the Proposed Spectrophotometric Method for Dacomitinib

Parameter	**Value**
Regression Equation	A = 0.0674x - 0.0056
Slope (S)	0.0674
Intercept (y-intercept)	-0.0056
Standard Deviation of Slope (SDs)	0.000905
Standard Deviation of Intercept (SDi)	0.008095
Correlation Coefficient (r)	0.9995
Determination Coefficient (R^2)	0.9991
Limit of Detection (LOD, µg/mL)	0.47
Limit of Quantification (LOQ, µg/mL)	1.4

Precision

High precision ensures that the method produces consistent results under the same conditions, which is important for the reliability of the analysis.

Repeatability (Intra-day): it is also known as intra-day precision. It was evaluated by determining DMB at specific concentrations on the same day with time interval of 2 hours for three times as shown in **Table 3**.

Intermediate precision (Inter-day): The Inter-day precision **Table 3** was studied by evaluating the prepared samples, for three consecutive days. The relative standard deviation (%RSD) was ranged from 0.001 to 0.034 %. This suggests the reproducibility, reliability and repeatability of the developed approach.

In comparison to other studies, our method's precision is consistent with or superior to previously reported methods. For instance,[29] reported intra-day and inter-day %RSD values of 0.42–1.23 % and 1.06–1.64%%, respectively, using a similar UV approach. The close agreement of our precision values with those in existing literature provides confidence in its reliability for accurate dacomitinib quantification.

Accuracy

High accuracy is crucial for ensuring that the measurement reflects the true concentration of the drug in the sample, which is vital for dosage formulation and therapeutic efficacy. Accuracy was determined via recovery studies, which require calculating the percentage mean recovery of the sample at three dissimilar concentrations. At each conc, three determinations were made. **Table 4** displays the resulting percent mean recovery. The accepted recovery limits are between 98% and 102%, and all the observed data fall within this range, indicating good recovery values and thus the method's accuracy.

The accuracy of the UV spectrophotometric method for dacomitinib analysis was evaluated by comparison with results obtained using a high-performance liquid chromatography (HPLC) method. Both results (section 3.9) were nearly identical, demonstrating a strong agreement between the two techniques. This close alignment supports the UV method's suitability as an accurate alternative to HPLC for dacomitinib quantification

When compared to other analytical methods reported in the literature, the accuracy of our UV method aligns closely with that of HPLC-based methods. For instance,[9] reported recovery rates of 99.7%–100.3% using HPLC, while another study[32] observed a mean recovery rate of 98.4-99.5% with a similar UV spectrophotometric approach. The slight differences in recovery values between our study and those in previous reports may be attributed to variations in sample preparation, solvent choice, or calibration techniques. Overall, our UV method demonstrates comparable accuracy, offering a reliable alternative to more complex and costly HPLC methods. The standard addition method was incorporated to enhance the accuracy and reliability of the validated analytical method **Table 5**.

Table 3: Results of Precision Studies for DMB.

	Concentration (ug/mL)	8	10	12
Precision	**Repeatability ± S.D.**			
	0 hr. (10:00 am)	101.25% ± 0.001	100% ± 0.001	99.1% ± 0.001
	2 hr. (12:00 pm)	98.75% ± 0.013	98.0% ± 0.021	98.3% ± 0.018
	4 hr. (2:00 pm)	98.75% ± 0.020	100% ± 0.003	99.1% ± 0.001
	Intermediate precision± S.D.			
	1st day	101.25% ± 0.018	98% ± 0.023	98.3% ± 0.017
	2nd day	102 % ± 0.034	101% ±0.034	100% ±0.011
	3rd day	98.75% ± 0.017	98.0% ± 0.023	99.1% ± 0.018

* Each value is the average of three separate determinations (n=3).

Table 4: Results of Accuracy studies for dacomitinib.

			Ethanol
Accuracy	At conc. Level 8µg/mL	% mean recovery± S.D	100.25 ± 0.45
		%RSD	0.45
	At conc. Level 10µg/mL	% mean recovery± S.D	100.50 ± 0.38
		%RSD	0.38
	At conc. Level 12µg/m	% mean recovery± S.D	99.75 ± 0.50
		%RSD	0.50

**** Each value is the average of three separate determinations (n=3).**

Table 5: Accuracy and Recovery studies by standard addition method at different concentration levels (4 ug/mL of Dacomitinib).

Accuracy level	**Amount of pure drug added (ug/mL)**	**Total amount of drug in theoretical value (ug/mL)**	**Experimental value (ug/mL)**	**%Assay content**	**±SD**	**% RSD**
75%	3	7	6.98	99.71%	0.045	0.65%
			6.92	98.86%		
			9.89	98.43%		
		Average	6.93	99.00%		
100%	4	8	7.92	99.0%	0.056	0.71%
			7.88	98.5%		
			7.81	97.63%		
		Average	7.87	98.38%		
125%	5	9	8.95	99.44%	0.076	0.86%
			8.88	98.67%		
			8.80	97.78%		
		Average	8.88	98.63%		

** Each value is the average of three separate determinations (n=3)**.**

Sensitivity

The proposed method's sensitivity for measuring DMB was proved to be reasonably high because LOD and LOQ were 0.47 and 1.4 µg/mL respectively, as calculated using the standard deviation method of Regression. LOD ensures that even very low concentrations of the drug can be detected, which is important in cases where the drug is present in trace amounts while LOQ ensures that low concentrations of the drug can be accurately quantified, which is important for precise dosage and formulation.

In comparison with prior research, our method demonstrates a competitive level of sensitivity. For example,[29] reported an LOD of 1.2 µg/mL and an LOQ of 3.7 µg/mL using a similar UV spectrophotometric technique. Another study for the UV spectrophotometric determination of dasatinib, member of the tyrosine kinase inhibitor family, the LOD and LOQ was 1.08 ug/mL and 3.29 ug/mL respectively[33] The slight differences observed may be attributed to variations in instrumental parameters, solvent systems, or wavelength settings. Notably, our method's sensitivity parameters fall within an acceptable range, reinforcing the robustness of the analytical approach used.

Robustness

The study assumed that any minor variations had no significant impact on the results, with recovery outcomes calculated as presented in **Table 6**. The robustness study assured the reliability of the developed method during normal analysis and proved that changing equipment illustrated non-significant effect. The low %RSD values observed in our study across different conditions reinforce the suitability of this UV method for dacomitinib, particularly in quality control settings where such variations may occur.

Table 6: Results of Robustness Study.

Concentration (ug/mL)	Absorbance Instrument 1	Absorbance Instrument 2	% Recovery
2	0.141	0.142	100.7%
4	0.251	0.24	95.6%
6	0.390	0.37	94.9%
8	0.541	0.51	94.3%
10	0.670	0.68	101.5%
12	0.800	0.80	100.0%
14	0.940	0.942	100.2%

**** Each value is the average of three separate determinations (n=3).**

Table 7: Summary of Validation Parameters for the Spectrophotometric Method of Dacomitinib.

Validation Parameter	**Results**	**Acceptance Criteria (ICH Guidelines)**
Linearity (µg/mL)	2 – 14 µg/mL	$r^2 \geq 0.999$
Regression Equation	y = 0.0674x – 0.0056	-
Correlation Coefficient (r^2)	0.9991	$r^2 \geq 0.999$
Accuracy (% Recovery)	99.75% – 100.5%	98% – 102%
Precision (% RSD)	%RSD for both intra-day and inter-day is 0.001-0.034%	RSD ≤ 2.0%
LOD (µg/mL)	0.47 µg/mL	Low enough to detect the analyte
LOQ (µg/mL)	1.4 µg/mL	Low enough for quantification
Robustness	No significant changes in results under small variations in parameters	Small variations should not affect results

Assay of Dacomitinib in Cubosomes nanoparticles

The developed spectrophotometric approach was used to directly determine DMB in Cubosomes without any sample extraction or filtration. The cubosomes were produced loading 10 mg of Dacomitinib. The Percentage mean recovery of DMB was determined using the studied spectrophotometric technique. The results were highly consistent with the amount of DMB loaded (Calculated % means recovery was 97%). The high percentage of DMB entrapment is very encouraging for further pharmaceutical investigation. The presence of a reliable analytical method of DMB determination in nanodrug delivery system will open a wide gate for exploiting the advantages of nanotechnology on the nascent drug. The SD and %RSD were ≤ 2, approving the correctness of method **Table 8**. This developed approach for dacomitinib determination encapsulated in Cubosomes was suggested to be easily applicable, with high validity, and good intra-day and inter-day precision.

Characterization of DMB loaded cubosomal dispersion

Entrapment Efficiency (EE%)

The% EE of DMB in cubosomes was 97.04 ± 0.30 % It was suggested that DM was incorporated into the cubosomal nanoparticle structure due to its strong affinity for the hydrophobic matrix of the nanoparticles. The high drug entrapment efficiency offers a significant advantage by enabling a therapeutic effect with a reduced dosage volume.[34,35]

Transmission electron microscope (TEM)

The photomicrograph **Fig. 5** of DMB-loaded cubosomal nanoparticles reveals cubic-shaped particles with the characteristic structural features of cubosomes. Additionally, the nanoparticles appear well-dispersed and distinct from one another, suggesting good stability.

Table 8: Validation sheet for quantification of Dacomitinib in Cubosomes nanoparticles, by applying the spectrophotometric method.

Parameter	Dacomitinib loaded cubosomes
Added amount (mg)	10.00
Recovered amount (mg)	9.67
%Average accuracy (±SD)	97 ± 0.0032
%RSD	0.0032
Repeatability (±SD)	100% ±0.011
intermediate Precision (±SD)	98.75% ± 0.017

* Each value is the average of three separate determinations (n=3).

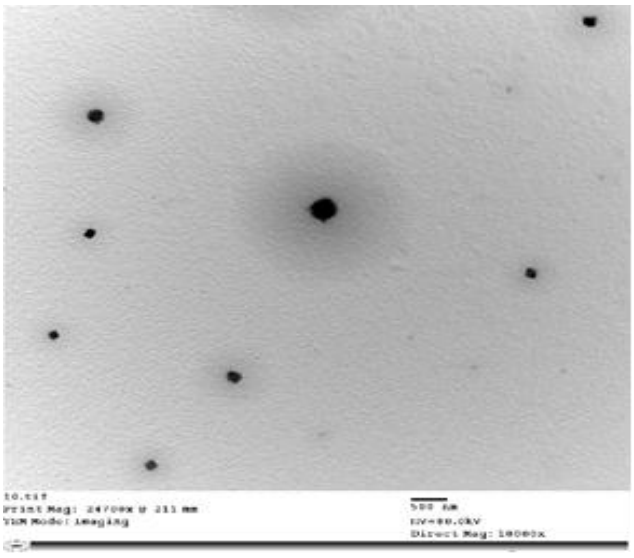

Fig. 5: TEM photomicrograph of DMB-loaded cubosomal nanoparticles.

Particle size, PDI and zeta potential

Cubosomal nanoparticles displayed a mean particle size of 203.4 ± 1.64 nm **Fig. 6**, PDI of 0.255± 0.01, zeta potential -34.80±1.06 mV **Fig. 7**. This PDI value is considered suitable for lipid-based drug delivery carriers, demonstrating uniform particle size distribution in the formulated dispersions with minimal aggregation.[36]

Selectivity Evaluation

The recorded spectra demonstrated no significant absorption at the λmax of Dacomitinib in placebo cubosomes and excipients, confirming minimal interference from formulation components. Similarly, spectral analysis of spiked plasma samples showed no substantial spectral overlap, indicating the method's specificity for Dacomitinib in complex biological matrices. These findings confirm the robustness of the method in distinguishing the analyte from excipients and endogenous components, ensuring its suitability for accurate quantification in pharmaceutical and biological samples.

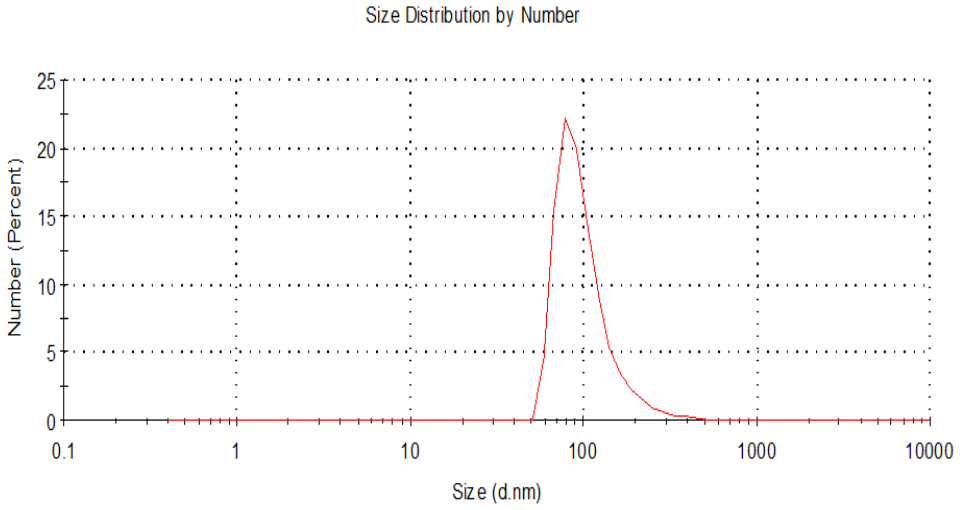

Fig. 6: Particle size of DMB loaded cubosomal dispersion.

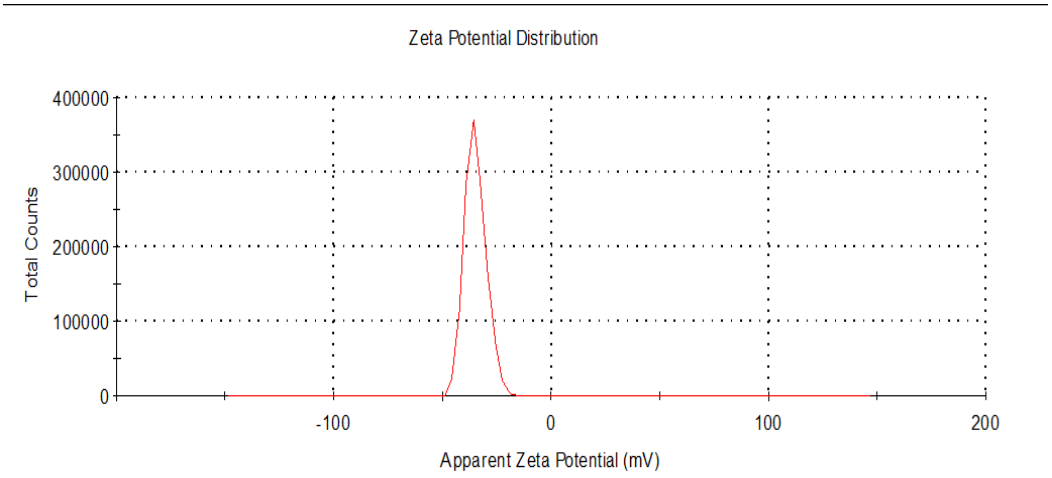

Fig. 7: Zeta potential of DMB loaded cubosomal dispersion.

HPLC determination as a reference standard method

Based on HPLC detection results, the ideal wavelength for determination of DMB was 254 nm with symmetrical drug peak shown in **Fig. 8**. The retention time was found to be 6.794 min. The Constructed calibration curve showed high linearly with R2 ≈ 0.999 **Fig. 9 and 10**. Applying the HPLC method for the drug quantification showed excellent recovery values (98–101.4%) with %RSD < 2 as illustrated in **Table 9**. The LOD and LOQ was 0.1208 ug/mL and 0.366 ug/mL respectively. The recovery of DMB from cubosomes was also accurate and precise, as evidenced by recovery experimental results in **Table 10**.

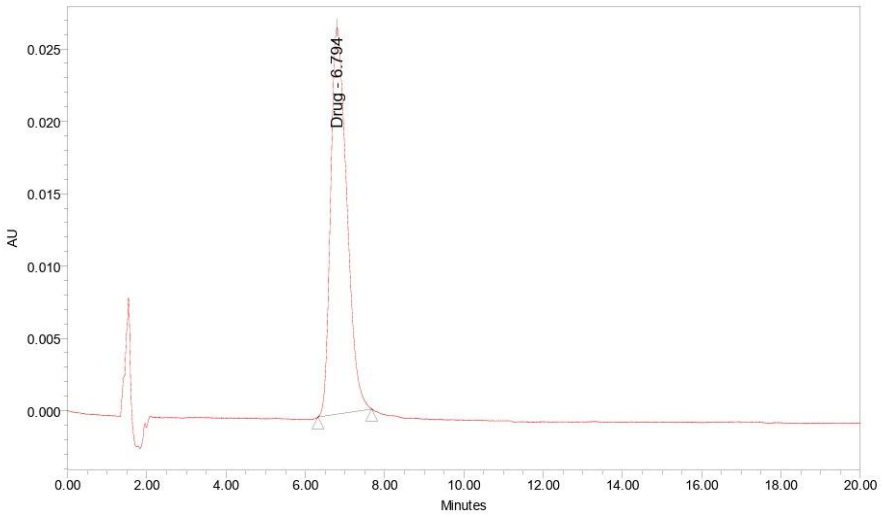

Fig.8: Representative HPLC chromatogram of Dacomitinib**.**

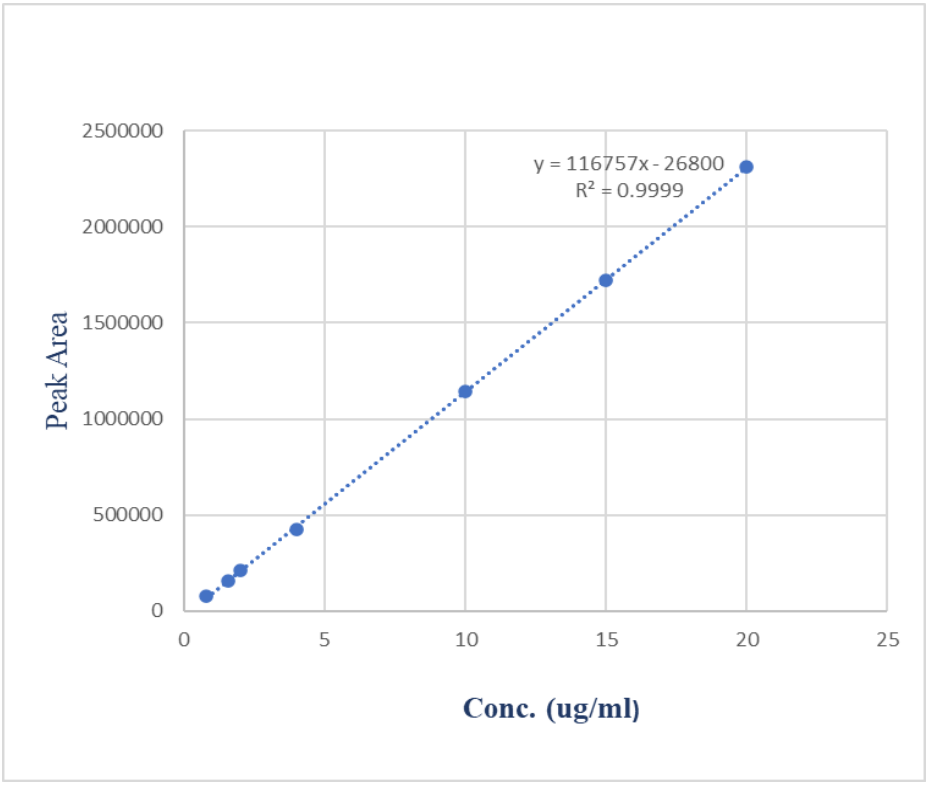

Fig.9: HPLC calibration Curve of dacomitinib.

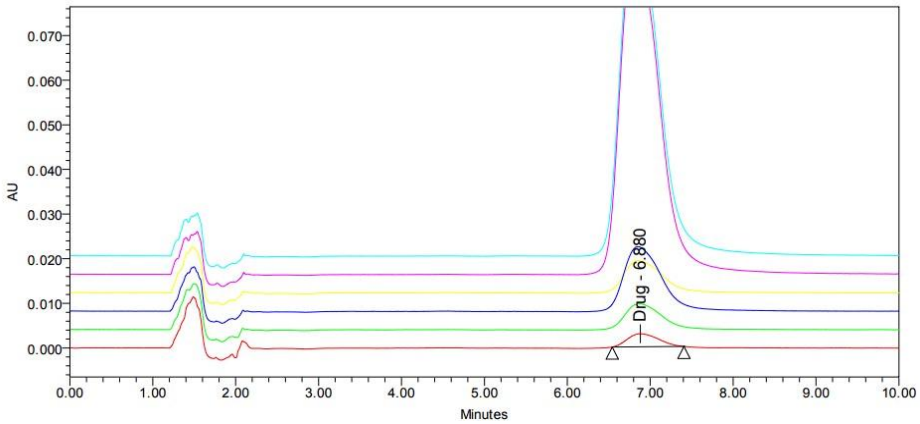

Fig.10: HPLC Chromatogram overlay of dacomitinib.

Table 9: Recovery experiments by HPLC for pure DMB.

Accuracy	At 8 ug/mL	% Mean recovery ± SD	99.87 ± 0.034
		%RSD	0.0336
	At 12 ug/mL	% Mean recovery ± SD	99.6 ± 0.023
		%RSD	0.023
Precision	Repeatability (%) ± SD		
	0 hr		100.1 ± 0.9
	2 hr		99.8 ± 0.8
	4 hr		99.7 ± 0.5
	Intermediate precision (%) ± SD		
	1st day		100.28 ± 1.1
	2nd day		100.03 ± 0.8
	3rd day		98.5 ± 0.8

** Each value is the average of three separate determinations (n=3).

Table 10: HPLC for estimating amount of dacomitinib in Cubosomes.

Parameter	DMB loaded cubosomes
added amount (mg)	10
Recovered amount (mg)	9.65
%Average accuracy (±SD)	98 ± 0.023
%RSD	0.023
Repeatability (±SD)	99.1% ± 0.001
intermediate Precision (±SD)	100.1% ± 0.011

Statistical analysis

The documented values of the percentage drug recovery from both the UV and HPLC methods were analyzed statistically by student paired t-test **Table 11**. NO noteworthy differences were detected in % drug recovery between the developed Spectrophotometric method and the HPLC method ($P < 0.05$, at 95% for level of significance). The new method confirmed a respectable accuracy when quantifying DMB in bulk and pharmaceutical dosage forms.

Assessment of the developed analytical method greenness

The proposed method was assessed using two widely recognized metrics: the Green Analytical Procedure Index (GAPI) and the Analytical GREEnness (AGREE).[37]

The results gained from GAPI matrix regarding the 15 parameters (pictogram) are

illustrated in **Fig. 11a** . Among all the 15 parameters, only 1 parameter (1) was given a red color in the pictogram. This is because samples collection/preparation was carried out in an offline manner. Yellow color was given to parameter (4) because the storage conditions require controlled environments (e.g., refrigeration) that consume energy but aren't overly intensive, parameter (5) because it is quantitative analysis, parameter (6) because the analysis was performed in microscale, parameter (7) because ethanol was used and parameter (14) indicating some waste is generated but in manageable quantities compared to high-waste methods. Meanwhile, green pictograms highlight the environmentally friendly aspects of the method. Consequently, this proposed method offers a straightforward procedure, minimizing waste production and reducing the use of hazardous substances.[38]

The AGREE pictogram is displayed in **Fig. 11b**. Parameter 1 (representing sample treatment) and Parameter 2 (representing sample amount) were marked yellow, as the sample was processed manually and used in moderate quantities. Parameter 3 (representing device positioning as either on-line or off-line) received a red color because the analysis was performed off-line. The remaining parameters were marked green, resulting in a total score of 0.82 out of 1, indicating a high level of greenness for the assay. Overall, the assessment tools validated the greenness of the proposed assay and its alignment with Green Analytical Chemistry (GAC) principles.[39]

Table 11: Statistical study of percentage drug recovery of the UV and HPLC methods.

	Pure DMB		DMB loaded Cubosomes
	8 ug/mL	12 ug/mL	
Spectrophotometric Method (% drug recovery)	99.58	98.83	97
HPLC Method (% drug Recovery)	99.87	99.6	98
Value of Significance (p<0.05)	0.053	0.028	0.027

* Each value is the average of three separate determinations (n=3).

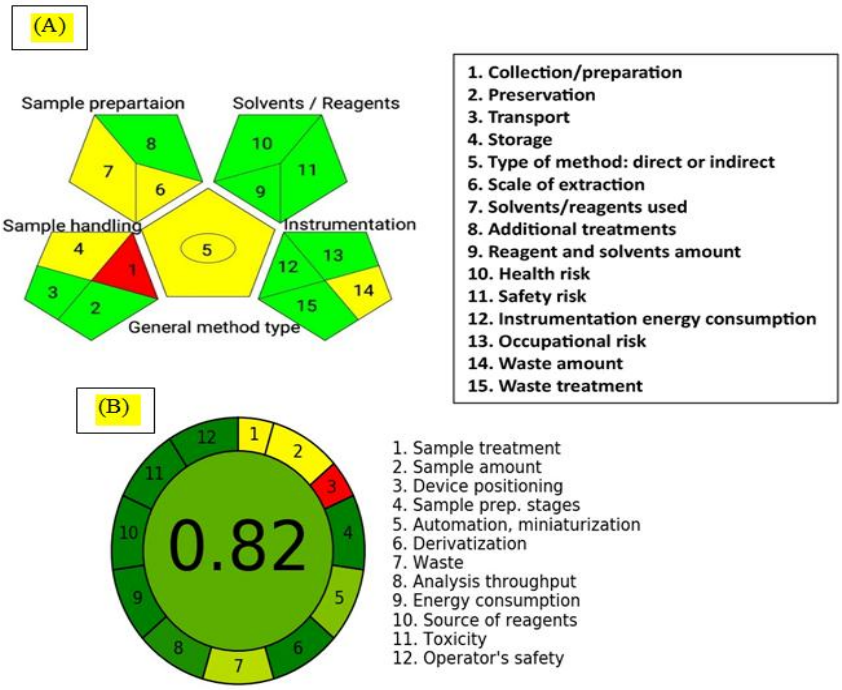

Fig. 11. a): GAPI assessment for evaluating the green profile of the proposed spectrophotometric method.
b): AGREE results for evaluating the greenness of the constructed method.

Conclusion

An easy, accurate, precise, valid, robust and First quick UV-spectrophotometric approach has been implemented to quantify DMB from its pharmaceutical formulations without any prior separation. Unlike the LC/MS procedure and the HPLC methods that needs high cost in hardware and reagents. The comparative study of the data revealed negligible significant differences between the drug quantities obtained by the spectrophotometer method and those estimated by HPLC approving the accuracy and precision of the developed method. The UV-visible spectrophotometer is a straightforward and reasonably priced piece of equipment, however due to its ease of use , simplicity, faster analysis, lower costs, simpler procedures, greater availability, biocompatibility, and environmental friendliness the method may be thought to be superior to those previously published and also other methods are costly, both in terms of initial investment and operational expenses, and requires skilled personnel for setup. The current approach is robust, durable, repeatable, accurate, and precise throughout a broad range and does not need any sophisticated techniques or instruments also shows an excellent green profile that has low impact on the environment.

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Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 335-353.

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تطبيق طريقة طيفية بالأشعة فوق البنفسجية لتقدير داكوميتينيب في شكله النقي وأنظمة توصيله النانوية: تقييم مدى صداقة الطريقة للبيئة باستخدام مقاييس معتمدة

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يُعد دواء داكوميتينيب (DMB) أحد أدوية العلاج الكيميائي التي تم اعتمادها مؤخرًا من قِبل إدارة الغذاء والدواء الأمريكية(FDA) ، مما يجعله مرشحًا جديدًا من المتوقع أن يكون محورًا للعديد من الأبحاث المستقبلية، خاصة في مجال تقنيات النانو. هدفت هذه الدراسة إلى تطوير طريقة اقتصادية وصالحة لقياس تركيز DMB في شكله النقي وفي الأشكال الصيدلانية المختلفة باستخدام طريقة الطيف المرئي–فوق البنفسجي .(UV-Visible Spectroscopy) تُعد الكيوبوسومات من النواقل النانوية المثيرة للاهتمام، حيث أظهرت دورًا فعالًا في توصيل أدوية العلاج الكيميائي إلى مواقع الأورام المختلفة، ولذلك تم اختيارها لاحتواء DMB لزيادة تحدي الدراسة.

تم قياس الطول الموجي الأقصى لـ DMB في مذيبات مختلفة، تلا ذلك إنشاء منحنيات الامتصاص لتحديد قيم الانحدار. ولمزيد من التحقق، تمت مقارنة طريقتنا المطورة إحصائيًا مع إحدى الطرق النادرة المتوفرة لتقدير DMB ، وهي طريقة الكروماتوغرافيا السائلة عالية الأداء (HPLC) لتقدير الدواء في شكله النقي أو عند احتوائه داخل الكيوبوسومات.

تم إجراء التحقق من صحة الطريقة وفقًا لإرشادات المؤتمر الدولي للتناغم .(ICH) أظهرت البيانات الناتجة أن الطريقة المطورة تمثل أسلوبًا اقتصاديًا للتحليل، دقيقًا ومتوافقًا مع توصيات إرشادات ICH، مما يتيح استخدامها مستقبلًا لتقدير الدواء. وقد تم تأكيد ذلك من خلال أن قيمة نسبة الانحراف المعياري النسبي (RSD) كانت أقل من ٢%، وتم تحقيق خطية جيدة في نطاق ٢–١٤ ميكروغرام/مل، وكانت حدود الكشف (LOD) والكمية 0.47 (LOQ) و١.٤ ميكروغرام/مل على التوالي.

تم تقييم صداقة الطريقة للبيئة باستخدام مقياسي GAPI وAGREE، مما يدل على إمكان استخدامها ضمن التطبيقات المستدامة بيئيًا .