RESEARCH PAPER

Development and Validation of a Stability-Indicating UV Spectrophotometric Method for Estimation of Sinapic Acid in Niosomal Nanoformulations

Simran Gawas[1], Vinayak Mastiholimath[1*]

[1]Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Basavan Kudachi, Belagavi- 591124, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

Corresponding author: Prof (Dr.) Vinayak Mastiholimath | Dean Faculty of Pharmacy and Head, Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy Belagavi, Basavan Kudachi, Belagavi- 591124, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, India | Email: mastiholimath@rediffmail.com

ORCID IDs: Simran Gawas - 0009-0004-6710-7790 | Vinayak Mastiholimath - 0000-0002-8560-4705

ABSTRACT

The present study was aimed at the development and validation of a stability-indicating UV spectrophotometric method for the estimation of Sinapic Acid and its application in niosomal nanoformulations using an eco-friendly analytical approach. A systematic experimental design was employed to optimize the analytical conditions and establish a simple, precise, and robust method. The developed method demonstrated excellent linearity, accuracy, precision, sensitivity, and robustness in compliance with ICH Q2 (R2) guidelines. Forced degradation studies carried out under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the stability-indicating capability of the method by effectively differentiating the intact drug from its degradation products. The validated UV spectrophotometric method was further successfully applied for the quantitative estimation of Sinapic acid in optimized niosomal nanoformulations, indicating its suitability for routine quality control analysis and analytical evaluation of niosomal drug delivery systems.

Keywords: Sinapic Acid, UV spectrophotometry, ICH Q2 (R2), stability-indicating method, niosomes, nanoformulations.

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1. Introduction

Cerebral neurons are essential for receiving, processing, storing, and transmitting information within the nervous system. Damage or degeneration of these neurons disrupts sensory, motor, and cognitive functions, ultimately impairing normal brain activity. Progressive neuronal dysfunction is a hallmark of neurodegenerative disorders, which lead to gradual and irreversible decline in cognitive and motor abilities [1]. Among these disorders, Alzheimer's disease (AD) is the most common cause of dementia worldwide. It is a chronic, progressive, age-associated neurological condition characterized by memory loss, cognitive deterioration, and reduced functional capacity. First described by Alois Alzheimer in 1906, AD prevalence increases significantly with age and is projected to rise substantially in coming decades [2].

The progression of AD is generally classified into four stages: pre-dementia, mild, moderate, and severe. In the early phase, episodic memory deficits are commonly observed, while motor and sensory functions remain relatively intact [3]. As the disease advances, memory impairment becomes more severe, particularly affecting recent memory and higher cognitive functions such as language, executive ability, and visuospatial skills [4]. At the molecular level, AD is associated with the accumulation of amyloid-β (Aβ) peptides in the brain. The disease is characterized by two major pathological features: extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). Amyloid plaques are mainly composed of aggregated

Aβ peptides, whereas NFTs consist of hyperphosphorylated tau protein, which disrupts neuronal cytoskeletal stability and function [5].Mutations in amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2) are strongly linked to familial early-onset AD. Sequential cleavage of APP by β-secretase and γ-secretase produces Aβ peptides, especially the highly aggregation-prone Aβ42 isoform. In addition, abnormal tau hyperphosphorylation causes its detachment from microtubules, impairing axonal transport and neuronal signaling, ultimately leading to neuronal degeneration and cell death. Dysregulation of kinase and phosphatase activity plays a significant role in the development of NFTs and AD pathology [6].

Sinapic acid (SA) is a naturally occurring hydroxycinnamic acid widely found in cereals, oilseeds, fruits, vegetables, and plants of the Brassicaceae family. Chemically known as 3,5-dimethoxy-4-hydroxycinnamic acid, it possesses strong antioxidant activity due to the presence of hydroxyl and methoxy functional groups. In recent years, SA has gained significant attention because of its diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, anticancer, neuroprotective, hepatoprotective, cardioprotective, and antidiabetic activities [7]. One of the important biological actions of SA is its ability to reduce oxidative stress by scavenging free radicals and reactive oxygen species (ROS). It also enhances endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while suppressing lipid peroxidation, thereby protecting cells from oxidative damage [8,9]. Several studies have reported the neuroprotective potential of SA in neurological disorders such as Alzheimer's disease, Parkinson's disease, epilepsy, and cerebral ischemia [10]. Its protective effects are mainly associated with antioxidant, anti-inflammatory, and anti-apoptotic mechanisms that help preserve neuronal integrity and cognitive function [11].

In Alzheimer's disease, SA has been shown to improve cholinergic neurotransmission by inhibiting acetylcholinesterase activity and increasing acetylcholine levels in the brain, which may enhance learning and memory [12]. Moreover, SA protects neurons against β-amyloid-induced toxicity, neuroinflammation, and mitochondrial dysfunction. Experimental studies in scopolamine-induced amnesia models have also demonstrated improvement in memory retention and cognitive performance following SA treatment [13]. Because of these multiple pharmacological actions, sinapic acid is considered a promising natural candidate for the prevention and management of neurodegenerative disorders, particularly Alzheimer's disease. However, further preclinical and clinical studies are still necessary to confirm its therapeutic efficacy and long-term safety.

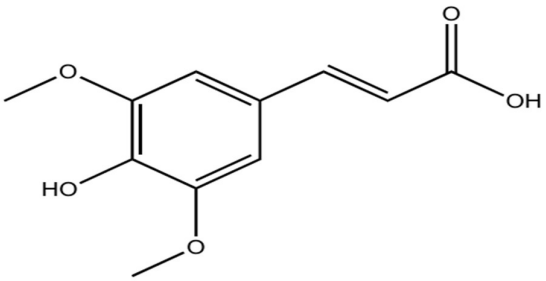


Figure 1 : Chemical Structure of Sinapic Acid

Ultraviolet–Visible (UV–Vis) spectroscopy is one of the most extensively utilized analytical techniques in pharmaceutical, chemical, biological, and material sciences for the qualitative and quantitative evaluation of compounds. Spectroscopy involves the study of the interaction between electromagnetic radiation and matter, wherein energy is absorbed or emitted in discrete packets known as quanta. The absorption of electromagnetic radiation provides significant information regarding the molecular structure, composition, and physicochemical properties of substances. UV–Vis spectroscopy specifically deals with the absorption of ultraviolet and visible regions of the electromagnetic spectrum by molecules, leading to electronic transitions between lower and higher energy states. Owing to its simplicity, sensitivity, rapidity, precision, and cost-effectiveness, UV–Vis spectroscopy has become an indispensable analytical tool in routine laboratory investigations and pharmaceutical analysis [14].

The principle of UV–Vis spectroscopy is primarily governed by the Beer–Lambert law, which states that the absorbance of a substance is directly proportional to its concentration and the path length of the absorbing medium. This relationship forms the basis for quantitative estimation of pharmaceutical compounds. The technique is widely employed for drug identification, assay determination, dissolution studies, stability testing, and quality control evaluations due to its reliability and reproducibility. Considering the growing therapeutic significance of Sinapic acid in the management of Alzheimer's disease, the development of reliable and validated analytical methods for its identification and quantification is of considerable importance. UV–Vis spectroscopy has emerged as a preferred analytical approach for the estimation of sinapic acid because of its operational simplicity, rapid analysis time, accuracy, and economical nature. The technique enables precise detection of sinapic acid through characteristic absorption peaks arising from electronic excitation within its aromatic and conjugated molecular structure. Furthermore, the obtained absorption spectra provide essential information concerning the concentration, purity, and structural characteristics of the analyte. Therefore, UV–Vis spectroscopic analysis plays a

crucial role in supporting formulation development, standardization, stability assessment, and quality control studies involving sinapic acid-containing pharmaceutical preparations.

2. Methodology

2.1. Materials

Sinapic acid was supplied by otto chemi privet, Limited. All additional chemicals and reagents employed in the study were of analytical grade and were procured from KLE College of Pharmacy.

2.2. Instrumentation

All spectrophotometric analyses were carried out using a Shimadzu UV-1900i Spectrophotometer integrated with LabSolutions software. Experimental optimization and statistical evaluation were performed using Design-Expert developed by Stat-Ease Inc.. Measurements were conducted at ambient room temperature, with double-distilled water used as the blank solvent throughout the analysis.

2.3. Selection of Solvent

Preliminary solubility studies were conducted using various solvents such as water, ethanol, methanol, and dimethyl sulfoxide (DMSO). Among these, methanol was found to provide superior solubility along with better spectral transparency and was therefore selected as the most suitable solvent for the analytical method development.

2.4. Preparation of Stock Solution

An accurately weighed quantity of Sinapic Acid (10 mg) was dissolved in methanol to prepare the primary stock solution. Subsequently, 1 mL of this solution was transferred into a 10 mL volumetric flask and diluted with a methanol–water mixture (60:40 v/v) to obtain the secondary stock solution. Further dilutions were carried out using distilled water to prepare working solutions in the concentration range of 2–10 µg/mL. The prepared solutions were sonicated for 5 minutes to ensure complete dissolution and uniformity.

2.5. Selection of Wavelength

The UV absorption spectra of the drug were recorded over the wavelength range of 200–400 nm using a UV–Visible spectrophotometer in order to determine the wavelength of maximum absorbance (λmax). Sinapic Acid showed maximum absorbance at 318 nm.

2.6. Method Validation

The optimized analytical method was validated in accordance with ICH Q2 (R1) guidelines. Validation parameters included specificity, selectivity, linearity, precision, accuracy, sensitivity, robustness, ruggedness, and repeatability [15].

2.6.1. Specificity and Selectivity

Specificity of the method was evaluated by examining potential interference from formulation excipients. Blank samples showed no notable absorbance at 318nm indicating that the method was selective and capable of accurately detecting drugs without interference.

2.6.2. Linearity

Linearity was determined over the concentration range of 2–10 µg/mL by plotting absorbance against concentration. Calibration curves were constructed, and regression equations along with correlation coefficient (R^2) values were calculated to verify the linear relationship between concentration and absorbance.

2.6.3. Precision

Precision of the developed method was assessed in terms of repeatability, intraday precision, and interday precision. Three different concentrations were analyzed in triplicate within the same day for intraday studies and on three successive days for interday studies. The reproducibility of the method was expressed using percentage relative standard deviation (%RSD).

2.6.4. Accuracy

Accuracy was determined by performing recovery studies at three levels corresponding to 80%, 100%, and 120% of the target concentration. The percentage recovery values obtained indicated the closeness of the measured concentrations to the actual drug content.

2.6.5. Sensitivity (LOD and LOQ)

The sensitivity of the method was estimated through the determination of the limit of detection (LOD) and limit of quantification (LOQ). These values were calculated based on signal-to-noise ratios of approximately 3:1 for LOD and 10:1 for LOQ.

2.6.6. Ruggedness

Ruggedness was examined by conducting the analysis using different analysts and separate instruments under similar experimental conditions. The obtained %RSD values confirmed the reproducibility and reliability of the method under variable laboratory conditions.

2.6.7. Robustness

Robustness of the analytical procedure was studied by introducing small deliberate variations in analytical parameters, specifically by changing the wavelength by ±2 nm from the λmax. The stability of the method against such minor changes was assessed using %RSD values.

2.6.8. Repeatability

Repeatability was evaluated by recording the absorbance of Sinapic acid six consecutive times at a concentration of 6 µg/mL under identical operating conditions. The %RSD values obtained

demonstrated the consistency and reliability of the developed method.

2.7. Forced Degradation Study

Forced degradation studies were performed to establish the stability-indicating capability of the developed UV spectrophotometric method. The drugs were subjected to various stress conditions, including acidic degradation (0.1 N HCl at 80°C for 2 h), alkaline degradation (0.1 M NaOH at 80°C for 2 h), oxidative degradation (30% H_2O_2 at 80°C for 2 h), and thermal degradation (40°C for 4 h). After treatment, the samples were diluted with water to obtain a final concentration of 10 µg/mL and scanned over the wavelength range of 200–400 nm. Considerable degradation was observed under the applied stress conditions, demonstrating clear spectral differentiation between degraded and intact drug substances [16,17].

2.8. Preparation of Niosomes

Niosomes were formulated using the ethanol injection technique with Span 60 and cholesterol serving as the primary vesicle-forming agents. The organic phase was prepared by dissolving Span 60, cholesterol, and the drug components in ethanol. Separately, the aqueous phase was heated to 60–70 °C and maintained under continuous stirring at 400 rpm. The organic solution was then added slowly in a dropwise manner into the aqueous phase. Stirring was continued for an additional 30 min to facilitate evaporation of ethanol and formation of vesicular dispersion. The resulting formulation was further sonicated to reduce vesicle size and subsequently stored in amber-colored containers at 4 °C until further use [18,19].

2.9. Application of the Method to Nanoformulation

The validated UV spectrophotometric method was successfully applied for the estimation of Simvastatin and Ezetimibe in the prepared niosomal formulation. An accurately measured quantity of the formulation was dispersed in water, sonicated thoroughly, and diluted appropriately. The prepared samples were then analyzed spectrophotometrically at their respective λmax values for drug quantification [20,21].

2.10. Greenness Assessment of the Developed Method

The environmental sustainability of the developed UV spectrophotometric method for Simvastatin and Ezetimibe was assessed using Complex GAPI, AGREE, and BAGI evaluation tools. The overall findings demonstrated that the proposed analytical method possesses low environmental impact while maintaining satisfactory analytical performance and practical utility.

Complex GAPI analysis was utilized to examine the ecological impact associated with different stages of the analytical procedure, such as sample preparation, solvent usage, instrumentation requirements, energy consumption, and waste production. The predominance of green and pale-yellow regions in the pictogram indicated reduced solvent hazards, minimal sample manipulation, lower energy requirements, and limited waste generation, thereby supporting the environmentally friendly nature of the method [22].

The AGREE assessment tool was further employed to determine the extent of compliance of the developed method with the twelve principles of Green Analytical Chemistry. AGREE generates a numerical greenness score ranging from 0 to 1 along with a circular graphical representation for easier interpretation. The obtained assessment confirmed that the developed UV spectrophotometric method exhibits a favorable green analytical profile due to its low solvent usage, simple operational procedure, reduced energy consumption, and use of uncomplicated instrumentation [23,24].

3. Results and Discussion

3.1. Selection of Solvent and Wavelength

Different solvents were investigated to identify the most suitable medium based on the solubility of the drug and the clarity of the obtained spectra. Among the tested solvents, water was selected as the optimized solvent system. Methanol was used as the blank throughout the spectrophotometric analysis. Both drugs were scanned over the wavelength range of 200–400 nm at a concentration of 10 µg/mL. Sinapic acid exhibited its maximum absorbance (λmax) at 318 nm.

3.2. Method Validation

Validation of an analytical method is a crucial step to ensure its reliability, accuracy, and suitability for the intended purpose. It is necessary to meet regulatory requirements, adhere to scientific guidelines, and maintain quality assurance standards. The optimized method was comprehensively validated in accordance with the recommendations of International Council for Harmonisation

3.2.1. Specificity and Selectivity

Selectivity of an analytical method refers to its ability to accurately determine the analyte in the presence of other components or potential interfering substances within the sample matrix. In the present study, the specificity of the developed method was established by the distinct absorbance maximum exhibited by Simvastatin at 318 nm. Furthermore, the method demonstrated good selectivity, as the solvent spectrum showed no interfering absorbance at the selected wavelength, confirming that the measurement of the analyte was not affected by the solvent system.

Study of Sinapic acid (n = 3)				
Concentration (µg/mL)	**%RSD**	**Day 1**	**Day 2**	**Day 3**
2	0.010	0.240	0.228	0.216
6	0.676	0.451	0.419	0.401
8	0.008	0.562	0.551	0.523

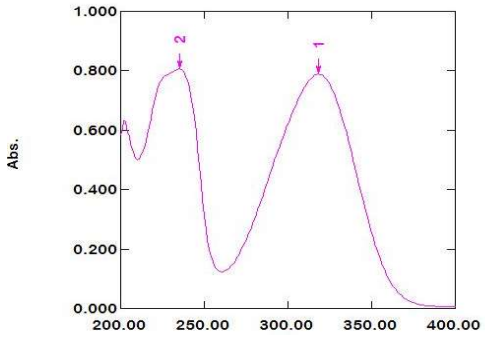
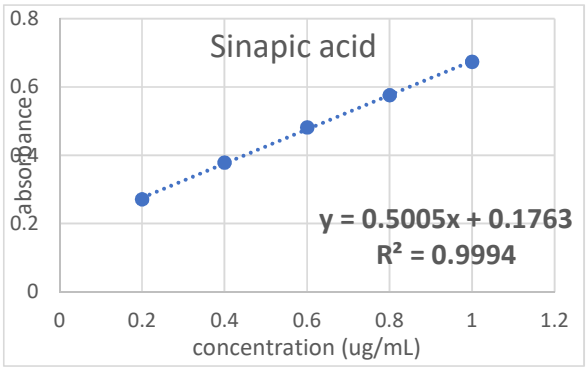


Figure.2. UV spectrum of Sinapic Acid

3.2.2. Linearity

Linearity of the analytical method refers to its ability to produce test results that are directly proportional to the concentration of the analyte within a defined range. In the present study, a strong linear correlation between concentration and absorbance was observed for Sinapic acid over the concentration range of 2–10 µg/mL. The calibration curve demonstrated excellent linearity with a regression coefficient (R^2) of 0.9994. Figure in 3 illustrates the calibration plot of Sinapic acid, confirming the direct proportional relationship between concentration and absorbance within the selected range.



Calibration Curve of Sinapic acid

Table 1. Linearity of Sinapic acid

3.2.3. System Precision

The method's precision was confirmed by evaluating both intraday and interday variability. Intraday precision was assessed by recording absorbance values at three different timesmorning, afternoon, and evening within the same day. Interday precision was measured by repeating the analysis across three successive days. The method showed strong precision, with all %RSD values remaining under 2%. Detailed results are provided in Tables 2 and 3.

Table 2. Intraday Precision

Table 3. Interday Precision

3.2.4. Accuracy

Accuracy reflects the closeness between the experimentally obtained values and the true values of the analyte. The percentage recovery of Sinapic acid

Sr. no.	Concentration (µg/ mL)	Absorbance of sinapic acid
1	0	0.000
2	2	**0.272**
3	4	**0.379**
4	6	**0.482**
5	8	**0.576**
6	10	**0.674**
R^2		**0.9994**
LOD		0.035 µg/mL
LOQ		0.035 µg/mL

was found to range between 97.31% and 100.08%, demonstrating the reliability of the developed analytical method. The consistent recovery values observed across different concentration levels confirmed the satisfactory accuracy and suitability of the method for analysis. The detailed recovery results are summarized in Table 4.

Table 4. Accuracy / Percentage Recovery Study

Study of Sinapic acid (n = 3)				
Concentration (µg/mL)	**%RSD**	**Morning**	**Afternoon**	**Evening**
2	0.10	0.340	0.321	0.311
6	0.004	0.553	0.525	0.404
8	0.003	0.725	0.729	0.715

Accuracy%	**Theoretical (µg/mL)**	**Actual (µg/mL)**	**%Recovery**

50%	6	6.927	97.61
100%	8	8.924	100.08
150%	10	8.826	97.31

3.2.5. Sensitivity

The sensitivity of the developed analytical method was evaluated by determining the limit of detection (LOD) and limit of 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, whereas the LOQ was determined using 10 times the same ratio. The calculated LOD and LOQ values for Sinapic acid were 0.035 µg/mL and 0.035 µg/mL, respectively. These findings indicate that the proposed method possesses excellent sensitivity and is capable of reliably detecting and quantifying Sinapic acid even at low concentrations.

3.2.6. Robustness and Ruggedness

Robustness of an analytical method reflects its capacity to remain unaffected by small but intentional variations in experimental conditions, thereby demonstrating the reliability of the procedure during normal laboratory operation. In the present study, robustness was evaluated by recording the absorbance at wavelengths of 320 ± 2 nm and 316 ± 2 nm.

Ruggedness represents the degree of reproducibility of results obtained under different analytical conditions, such as analysis performed by different analysts. The ruggedness of the developed method was assessed by two independent analysts using sample concentrations of 2, 6, and 10 µg/mL, and the corresponding absorbance values were measured.

The low percentage relative standard deviation (%RSD) values obtained for repeatability, reproducibility, and robustness studies, all being below 2%, confirmed that the developed method is precise, reliable, and unaffected by minor experimental variations. The results obtained for ruggedness and robustness are summarized in Tables 5 and 6, respectively.

Table.5. Ruggedness Study with Change in Analyst

Concentration (µg/mL)	Analyst 1 (%RSD)	Analyst 2 (%RSD)
2	0.201	0.440
6	0.517	0.562
8	0.156	0.174

Table.6. Robustness Study with Change in Wavelength

Concentration (µg/mL)	316 nm (%RSD)	320 nm (%RSD)
2	0.478	1.01
6	0.147	0.587
8	0.583	0.255

3.2.7. Repeatability

Repeatability, also referred to as intra-assay precision, describes the capability of an analytical method to produce consistent and reproducible results when performed repeatedly under identical operating conditions within a short time interval. A percentage RSD value below 2% indicates good precision and confirms the satisfactory repeatability of the developed method. The obtained results are summarized in Table 7

Table 7.

Forced degradation assay	
	Sinapic acid
Acidic	7.34%
Basic	16.51%
Oxidative	9.81%
photolytic	21.57%
Thermal	13.94%

Repeatability Study of the Developed Method (n = 6)

3.2.8. Forced Degradation Study

The forced degradation study revealed that the drug was susceptible to degradation under acidic, alkaline, oxidative, and photolytic stress conditions. Among these, maximum degradation was observed in oxidative and alkaline environments. The results established the stability-indicating capability of the developed method (Table 8).

Formulation	Particle Size(nm)	PDI	Zeta Potential(mV)
Sinapic acid - loaded niosomes	132	0.221	-20.17

Table 8. Results of Forced Degradation Study

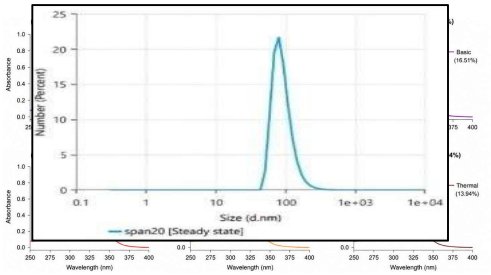


Figure. 4. a) Acidic b) Basic c) oxidative d) photolytic, Forced Degradation assay of Sinapic acid

3.2.9. Estimation of Sinapic Acid in Niosomes

With the growing development of nanocarrier-based drug delivery approaches, the need for accurate and dependable analytical techniques for formulation evaluation has become increasingly important. Precise determination of the drug entrapped within niosomal systems plays a vital role in ensuring formulation quality, stability, and therapeutic effectiveness. In the present investigation, a validated UV spectrophotometric method was developed and successfully employed for the quantitative estimation of Sinapic acid in the prepared niosomal formulation.

Table 9. Estimation and Characterization of niosomes Nanoformulation

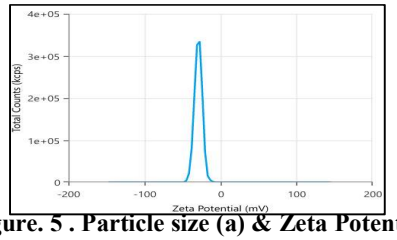


Figure. 5 . Particle size (a) & Zeta Potential (b) of the Optimized formulation

3.3. Analysis of Transmission Electron Microscopy

Transmission electron microscopy (TEM) images revealed the formation of predominantly spherical niosomal vesicles with a uniform distribution and well-defined boundaries. The vesicles were observed within the nanometric size range and exhibited minimal aggregation, indicating successful formation and good stability of the niosomal system.

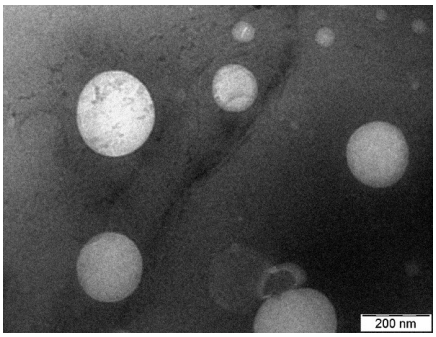


Figure. 6. Analysis of Transmission Electron Microscopy

3.4. Assessment of Method Greenness and Whiteness Attributes:

The environmental sustainability of the developed UV spectrophotometric method for the simultaneous estimation of Simvastatin and Ezetimibe was evaluated using the Complementary Green Analytical Procedure Index (ComplexGAPI). The obtained pictogram (Fig. 7.a) consisted of seven green, nine yellow, and one red section, suggesting that the method possesses acceptable environmental characteristics. The green areas indicated the use of relatively safer solvents and reduced solvent consumption, whereas the yellow areas were mainly related to sample preparation steps and the energy requirements of the analytical instrument. In addition, the central E-factor value of 1.0 demonstrated minimal waste production, highlighting the method's compliance with the principles of green analytical chemistry.

The eco-friendliness of the proposed UV spectrophotometric method was also examined using the Analytical GREEnness (AGREE) assessment tool, which measures conformity with the twelve principles of green analytical chemistry. The developed method achieved an AGREE score of 0.8, and the generated pictogram (Fig. 7.b) showed a predominantly green appearance, indicating excellent environmental compatibility and low ecological burden.

Further evaluation of method whiteness was carried out using the Blue Applicability Grade Index (BAGI), an assessment tool that integrates analytical efficiency, environmental impact, and practical usability into a unified metric. The developed method attained a BAGI score of 70, reflecting a favorable balance between analytical reliability, sustainability, and operational applicability. These findings support the suitability of the UV spectrophotometric method for routine estimation of Simvastatin and Ezetimibe in pharmaceutical analysis (Fig. 7.c).

Overall, the greenness and blueness evaluation results demonstrate that the proposed method effectively combines analytical accuracy with environmental sustainability and practical efficiency.

Owing to its low solvent consumption, reduced waste generation, and reliable performance, the method can be considered appropriate for routine high-throughput applications in pharmaceutical quality control and regulatory laboratories.

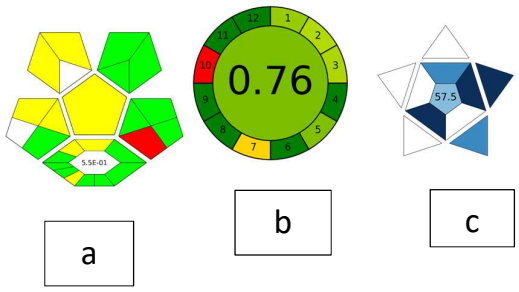


Figure. 7. Assessment of Method Greenness and Whiteness

4. Conclusion

The present study successfully developed and validated a simple, sensitive, accurate, and environmentally friendly UV spectrophotometric method for the estimation of Sinapic acid. The method demonstrated excellent linearity, precision, accuracy, robustness, ruggedness, repeatability, and sensitivity in accordance with ICH guidelines. Forced degradation studies confirmed the stability-indicating capability of the method under various stress conditions. Furthermore, the developed method was effectively applied for the quantitative estimation of Sinapic acid in niosomal formulations, confirming its suitability for nanoformulation analysis. TEM analysis revealed the successful formation of stable spherical niosomal vesicles with uniform distribution. Greenness assessment using Complex GAPI, AGREE, and BAGI tools established the eco-friendly and sustainable nature of the analytical procedure. Overall, the developed method can be considered reliable, economical, and suitable for routine pharmaceutical quality control and formulation studies involving Sinapic acid.

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Author Contributions

Author 1 contributed to conceptualization, investigation, methodology, software handling, and preparation of the original draft manuscript. Author 2 was involved in project administration, supervision, and manuscript review and editing.

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Disclosure of Conflicts of Interest

The authors declare that there are no conflicts of interest or financial relationships that could have influenced the outcomes or interpretations of this study.

Use of Artificial Intelligence (AI)-Assisted Technology

The authors confirm that no artificial intelligence (AI)-assisted tools were utilized in the writing, editing, or preparation of this manuscript, and no AI-generated or AI-modified images were included in this work.

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