

Development, Validation, and Greenness Assessment of a UV Spectrophotometric Method for Simultaneous Estimation of Thiocctic Acid and Sesamol

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ABSTRACT

A simple, rapid, accurate, and eco-friendly UV spectrophotometric method was developed and validated for the simultaneous estimation of Thiocctic acid (TCA) and Sesamol (SML) in liposomal formulations. The method was based on the measurement of absorbance at 333 nm for TCA and 298 nm for SML using a methanol as a solvent. The developed method exhibited excellent linearity over the concentration range of 2–10 µg/mL with correlation coefficients (R^2) of 0.999 for TCA and 0.998 for SML. The method demonstrated satisfactory precision, with intraday and interday %RSD values below 2%. Accuracy studies showed percentage recoveries ranging from 99.77–101.08% for TCA and 99.18–100.95% for SML. The limits of detection and quantification were found to be 0.221 and 0.671 µg/mL for TCA, and 0.272 and 0.824 µg/mL for SML, respectively, indicating good sensitivity. Robustness, ruggedness, and repeatability studies further confirmed the reliability of the method. Forced degradation studies under acidic, alkaline, oxidative, and photolytic conditions demonstrated the capability of the method to monitor drug degradation, with notable degradation observed under oxidative and sunlight stress conditions. The validated method was successfully applied for the simultaneous estimation of TCA and SML in liposomal formulations. Furthermore, greenness assessment using ComplexGAPI, AGREE, and BAGI tools confirmed the environmental sustainability, operational simplicity, and analytical efficiency of the developed method. Therefore, the proposed UV spectrophotometric method can be effectively employed for routine quality control analysis of Thiocctic acid and Sesamol in pharmaceutical formulations.

Keywords: Thiocctic acid, Sesamol, UV spectrophotometry, Method validation, Liposomes, Greenness assessment, AGREE, BAGI, ComplexGAPI.

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1. Introduction

Hemorrhoidal disease is one of the most common anorectal disorders affecting millions of people worldwide, with an estimated global prevalence ranging from 4% to 40% depending on lifestyle, dietary habits, and age group. The condition is characterized by swelling and inflammation of the vascular structures present in the anal canal and is commonly associated with symptoms such as pain, bleeding during defecation, itching, irritation, prolapse, and discomfort. Factors including chronic constipation, prolonged straining, obesity, sedentary lifestyle, and pregnancy contribute to its development. Hemorrhoids significantly affect patient quality of life and may require medical or surgical management based on disease severity.[1-4] Sesamol (SEL), chemically known as 3,4-methylenedioxyphenol, is a naturally occurring phenolic compound obtained from sesame seeds and

sesame oil of *Sesamum indicum*. [5] It possesses antioxidant, anti-inflammatory, antibacterial, and antifungal properties due to the presence of hydroxyl and methylenedioxy functional groups in its structure [6]. Thiocctic acid, also known as alpha-lipoic acid, is an eight-carbon organosulfur fatty acid containing an intramolecular disulfide bond [7]. It exhibits both hydrophilic and lipophilic characteristics because of the presence of a carboxylic acid group and hydrocarbon chain. Both compounds possess significant antioxidant and anti-inflammatory activities, making them promising therapeutic agents for inflammatory disorders [8,9]. Although various analytical methods have been reported for the individual estimation of Thiocctic acid and Sesamol using techniques such as UV-visible spectrophotometry, HPLC but no validated UV spectrophotometric method has been reported for their simultaneous estimation in combined formulation. UV spectrophotometry offers

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advantages including simplicity, rapid analysis, cost-effectiveness, and minimal solvent consumption, making it suitable for routine quality control analysis. Therefore, the present study was undertaken to develop and validate a simple, accurate, precise, and stability-indicating UV spectrophotometric method for the simultaneous estimation of Thiocctic acid and Sesamol in the developed Liposomes formulation.

The present study was focused on the development and validation of a simple, precise, accurate, robust, and economical UV spectrophotometric method for the simultaneous estimation of Thiocctic acid and Sesamol and to evaluate its applicability in the analysis of the developed liposomal formulation. The developed method may serve as a reliable analytical tool for routine quality control and standardization of the formulation.

2. Methodology

2.1. Materials:

Thiocctic acid and Sesamol were procured from Otto Chemie Pvt. Ltd. All other chemicals, solvents, and reagents used in the study were of analytical grade and were obtained from KLE College of Pharmacy.

2.2. Instrumentation:

All spectrophotometric analyses were carried out using a Shimadzu Corporation UV-1900i UV-visible spectrophotometer integrated with Lab Solutions software. Experimental optimization and data analysis were performed employing Design-Expert software (Version 13.0, Stat-Ease Inc., Minneapolis, USA). Double-distilled water was utilized as the blank solution for all measurements, and the studies were conducted under ambient laboratory conditions.

2.3. Selection of Solvent

Various solvents such as water, ethanol, methanol, and dimethyl sulfoxide (DMSO) were screened during the preliminary investigation. Among them, water was selected as the most suitable solvent based on satisfactory solubility and clear spectral characteristics observed during analysis.

2.4. Preparation of Stock Solution:

Accurately weighed amounts of Thiocctic acid and Sesamol (10 mg each) were transferred into a volumetric flask and dissolved in methanol to prepare the primary stock solution. Subsequently, 1 mL of this solution was pipetted and further diluted to 10 mL using a methanol to obtain the secondary stock solution. Appropriate dilutions were then carried out with water to prepare working standard solutions within the concentration range of 2–10 µg/mL. The prepared solutions were sonicated for 5 minutes to ensure complete mixing and uniform dissolution.

2.5. Selection of Wavelength:

Thiocctic acid and Sesamol were scanned in the wavelength range of 200–400 nm using UV spectrophotometry to determine their maximum

absorbance wavelengths (λ max). Thiocctic acid exhibited λ max at 333 nm, whereas Sesamol showed λ max at 298 nm.

2.6. Method Development and Optimization

Method development was carried out using a systematic approach by selecting suitable analytical conditions for simultaneous estimation of Thiocctic acid and Sesamol. UV spectrophotometry was chosen as a simple, rapid, sensitive, and cost-effective analytical technique for quantitative analysis. The absorbance response of both drugs was considered as the critical analytical parameter during method optimization. Different solvent systems were evaluated to obtain clear solutions and stable spectral characteristics. The standard drug solutions were scanned in the wavelength range of 200–400 nm to determine the absorption maxima of both analytes. Thiocctic acid exhibited maximum absorbance at 333 nm, whereas Sesamol showed maximum absorbance at 298 nm. The optimized analytical conditions provided good absorbance response, sensitivity, and reproducibility for simultaneous estimation of both drugs [10,11].

2.7. Method Validation

The developed UV spectrophotometric method was validated in accordance with ICH Q2 (R1) guidelines by evaluating parameters such as specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), ruggedness, robustness, and repeatability. The validation studies confirmed that the method was reliable, accurate, and suitable for routine quantitative analysis of Thiocctic acid and Sesamol [12].

2.7.1. Specificity and Selectivity

Specificity of the developed method was evaluated by examining the interference from formulation excipients and blank solutions. No noticeable absorbance interference was observed at the selected analytical wavelengths of 298 nm and 333 nm, demonstrating the selective nature of the method for simultaneous estimation of both drugs [13].

2.7.2. Linearity

Linearity of the method was established by preparing calibration solutions within the concentration range of 2–10 µg/mL. Calibration curves were plotted between concentration and absorbance, and the corresponding regression equations along with correlation coefficient (R^2) values were determined to confirm the linear relationship [14].

2.7.3. Precision

Precision of the analytical method was assessed in terms of repeatability, intraday precision, and interday precision. Different concentrations of the analytes were analyzed in triplicate within the same

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day and on three consecutive days. The obtained results were expressed as percentage relative standard deviation (%RSD) to evaluate the reproducibility of the method.

2.7.4. Accuracy

Accuracy of the developed method was determined by recovery studies performed at 80%, 100%, and 120% concentration levels. Known amounts of standard drug were added to the pre-analyzed sample, and the percentage recovery was calculated to determine the closeness between the measured and actual values [15].

2.7.5. Sensitivity (LOD and LOQ)

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

2.7.6. Ruggedness

Ruggedness of the method was evaluated by carrying out the analysis using different analysts and instruments under similar experimental conditions. The %RSD values obtained from the analysis confirmed the reproducibility of the method under varied laboratory environments.

2.7.7. Robustness

Robustness studies were performed by introducing small deliberate changes in analytical parameters, particularly wavelength variation of ± 2 nm from the selected λ_{max} values. The effect of these changes on absorbance was evaluated using %RSD values, indicating the stability and reliability of the developed method.

2.7.8. Repeatability

Repeatability of the method was assessed by analyzing six replicates of standard drug solutions at a concentration of 8 $\mu\text{g/mL}$ under identical operating conditions. The low %RSD values obtained demonstrated the consistency and precision of the developed analytical method.

2.8. Forced Degradation Study

Forced degradation studies were performed to evaluate the stability-indicating capability of the developed UV spectrophotometric method. The analytes were subjected to different stress conditions including acidic hydrolysis (0.1 N HCl at 80°C for 2 h), alkaline hydrolysis (0.1 M NaOH at 80°C for 2 h), oxidative degradation (30% H₂O₂ at 80°C for 2 h), and thermal degradation (40°C for 4 h).

Following stress treatment, the degraded samples were diluted to obtain a final concentration of 10 $\mu\text{g/mL}$ using distilled water and scanned within the wavelength range of 200–400 nm. Noticeable degradation was observed under the applied stress

conditions, while clear spectral distinction between degraded and non-degraded drugs confirmed the stability-indicating nature of the developed method [16,17].

2.3. Preparation of Liposomes

TCA and SML-loaded liposomes were prepared using the ethanol injection method. Soya lecithin and cholesterol in a 5:1 molar ratio were dissolved in ethanol and sonicated to obtain a homogeneous solution, followed by addition of TCA and SML. The organic phase was injected dropwise into purified water maintained at 50 °C under magnetic stirring. The obtained dispersion was allowed to hydrate at room temperature and further homogenized using Ultra-Turrax homogenizer and probe sonication. The prepared liposomal dispersion was stored at 4 °C and characterized for particle size, polydispersity index, and zeta potential using Zetasizer Nano ZS [18].

2.10. Application of the Method to Nanoformulation

The validated UV spectrophotometric method was successfully utilized for the quantitative estimation of Thioctic acid and Sesamol in the developed nanoformulation. An accurately measured quantity of the formulation was dispersed in a suitable volume of distilled water and subjected to sonication to ensure complete extraction of the drugs from the vesicular system. The resulting solution was filtered, suitably diluted, and analyzed using a UV-visible spectrophotometer at their respective analytical wavelengths. The developed method demonstrated satisfactory applicability for routine estimation of both analytes in the nanoformulation.

2.11. Greenness Assessment of the Developed Method

The environmental sustainability and eco-friendly nature of the developed UV spectrophotometric method for simultaneous estimation of Thioctic acid and Sesamol were systematically evaluated using modern green analytical assessment tools, namely Complex Green Analytical Procedure Index (Complex GAPI), Analytical GREENness Metric Approach (AGREE), and Blue Applicability Grade Index (BAGI). These assessment approaches were employed to determine the environmental impact, analytical efficiency, operational safety, and overall sustainability profile of the developed analytical procedure [19].

Complex GAPI assessment was performed to examine the ecological impact of each stage involved in the analytical procedure, including sample preparation, solvent usage, instrumentation, energy consumption, and waste production. The predominance of green and light-yellow regions in the pictogram reflected the eco-friendly nature of the

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developed analytical procedure with reduced hazardous solvent consumption and minimal waste generation.[20]

The AGREE tool was employed to determine the degree of compliance of the method with the twelve principles of Green Analytical Chemistry. The generated AGREE score and visual representation confirmed that the developed UV method is environmentally favorable due to its low solvent requirement, simple instrumentation, and minimal energy utilization.[21]

Furthermore, the Blue Applicability Grade Index (BAGI) was used to evaluate the overall sustainability profile of the analytical method by integrating environmental impact, analytical efficiency, operational simplicity, and user safety.[22]

The obtained BAGI score demonstrated that the developed method achieved an appropriate balance between analytical reliability and environmental sustainability. Overall, the greenness assessment established that the proposed UV spectrophotometric method is eco-friendly, efficient, and suitable for routine analysis of Thiocetic acid and Sesamol in nanof ormulation systems.

3. Results and Discussion

3.1. Selection of Solvent and Wavelength

A mixture of methanol and water in the ratio of 50:50 (v/v) was selected as the suitable solvent system after evaluating different solvents based on drug solubility and spectral clarity. All experimental measurements were performed using methanol as the blank. Thiocetic acid and Sesamol were scanned in the wavelength range of 200–400 nm at a concentration of 10 µg/mL. Thiocetic acid exhibited maximum absorbance at 333 nm, whereas Sesamol showed maximum absorbance at 298 nm.

3.2. Method Development

A robust UV spectrophotometric method was developed for the simultaneous estimation of Thiocetic acid and Sesamol. Different analytical conditions were evaluated to obtain accurate, precise, and reliable results. Absorbance was selected as the analytical response parameter because it directly reflects the concentration of the analytes.

3.3. Method Validation

Validation of the analytical method was carried out to ensure the reliability, accuracy, precision, and suitability of the developed UV spectrophotometric method for simultaneous estimation of Thiocetic acid and Sesamol. The optimized method was validated in accordance with ICH Q2 (R1) guidelines.

3.3.1. Specificity and Selectivity

The specificity and selectivity of the developed UV spectrophotometric method were evaluated to ensure accurate estimation of Thiocetic acid and Sesamol in the presence of other components. Thiocetic acid and Sesamol exhibited maximum absorbance at 333 nm and 298 nm, respectively. The selectivity of the method was confirmed by the absence of interfering absorbance from the solvent system at the selected wavelength, indicating that the method was specific for simultaneous estimation of both drugs.

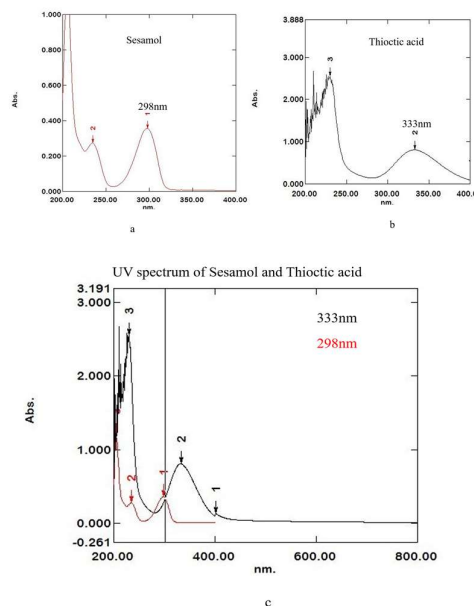


Figure 1. a: UV spectrum of Sesamol, b: UV spectrum of Thiocetic acid C: UV Spectrum of Sesamol and Thiocetic acid.

The UV spectrum shown in Figure 1 a, b, c, demonstrated that Sesamol exhibited maximum absorbance at 298 nm, whereas Thiocetic acid showed maximum absorbance at 333 nm. The observed wavelengths were found to be in agreement with the reported literature values.

3.4.2. Linearity

Linearity of the developed UV spectrophotometric method was evaluated by determining the relationship between concentration and absorbance of Thiocetic acid and Sesamol. A linear response was observed within the concentration range of 2–10 µg/mL for both drugs. The regression coefficient values were found to be 0.999 for Thiocetic acid and 0.998 for Sesamol, indicating good linearity of the method. Figures 2C and 2D represent the calibration curves of Thiocetic acid at 333 nm and Sesamol at 298 nm, respectively, demonstrating a direct relationship between concentration and absorbance within the selected concentration range (Table 1 and Figures 2C and 2D).

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Table 1. Linearity of Thiocctic acid and Sesamol

Sesamol Intraday precision (n=3)				
Concentration (µg/ml)	%RSD	Morning	Afternoon	Evening
2	0.46	0.125	0.124	0.124
6	0.47	0.365	0.365	0.368
10	0.25	0.603	0.600	0.602
Thiocctic acid Intraday precision (n=3)				
Concentration (µg/ml)	%RSD	Morning	Afternoon	Evening
2	1.69	0.143	0.144	0.142
6	0.21	0.462	0.461	0.460
10	0.21	0.711	0.709	0.712

Sr. no.	Concentration (µg/mL)	Absorbance of Sesamol (298)	Absorbance of Thiocctic acid (333)
1	0	0.0	0.0
2	2	0.125	0.143
3	4	0.247	0.300
4	6	0.365	0.462
5	8	0.488	0.561
6	10	0.603	0.711
R ²		0.998	0.999
LOD		0.272 µg/mL	0.221 µg/mL
LOQ		0.824 µg/mL	0.671 µg/mL

Table 2. Intraday precision assay

Table 3. Interday precision assay

Sesamol Interday precision (n=3)				
Concentration (µg/ml)	%RSD	Day 1	Day 2	Day 3
2	1.07	0.124	0.125	0.126
6	0.476	0.365	0.365	0.368
10	0.21	0.603	0.605	0.600

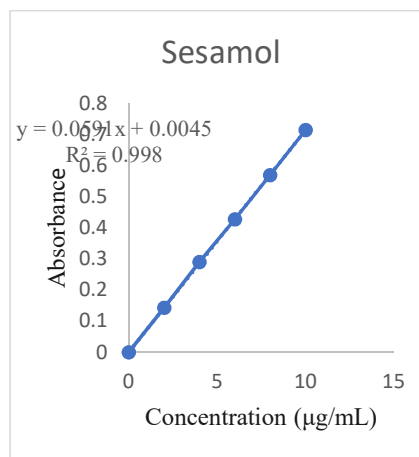


Figure . 2: Calibration curve of Sesamol

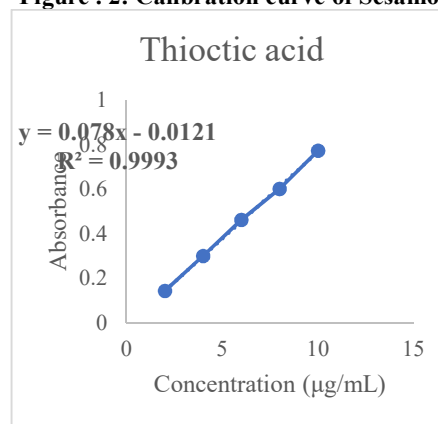


Figure.3. Standard Calibration Curve of Thiocctic acid

3.4.3. System Precision

The precision of the developed UV spectrophotometric method was evaluated by performing intraday and interday studies. Intraday precision was determined by measuring the absorbance of the analytes at three different time intervals within the same day, namely morning, afternoon, and evening. Interday precision was assessed by repeating the analysis over three consecutive days. The method exhibited good precision, as all percentage relative standard deviation (%RSD) values were found to be below 2%, indicating satisfactory reproducibility of the method. The obtained results are presented in Tables 2 and 3.

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Thioctic acid Interday precision (n=3)				
Concentration (µg/ml)	%RSD	Day 1	Day 2	Day 3
2	0.80	0.143	0.140	0.141
6	0.47	0.462	0.464	0.461
10	0.41	0.711	0.709	0.712

3.4.4. Accuracy

The accuracy of the developed UV spectrophotometric method was evaluated by recovery studies at different concentration levels. The percentage recovery of Thioctic acid was found to be in the range of 99.7–101.08%, whereas Sesamol showed recovery values between 99.18–100.9%. The consistent recovery results obtained at various concentration levels indicated that the developed method possesses good accuracy and reliability for simultaneous estimation of both drugs. The detailed recovery data are presented in Table 4.

Table 4. Accuracy/percentage recovery

LEVEL	Concentration (µg/ml)	Absorbance of SML (298 nm)	% Recovery	Absorbance of TCA (333 nm)	% Recovery
80%	4	0.247	100.95%	0.300	99.77%
		0.246		0.302	
		0.246		0.301	
100%	6	0.365	99.18%	0.462	101.08%
		0.370		0.478	
		0.362		0.475	
120%	8	0.488	100.48%	0.561	99.87%
		0.486		0.568	
		0.485		0.559	

3.4.5. Sensitivity

The sensitivity of the developed UV spectrophotometric 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 calculated using 10 times the

same ratio. The obtained LOD and LOQ values for Thioctic acid were found to be 0.221 µg/mL and 0.671 µg/mL, respectively. Similarly, the LOD and LOQ values for Sesamol were observed to be 0.272 µg/mL and 0.824 µg/mL, respectively. The low LOD and LOQ values indicated that the developed method possesses good sensitivity for simultaneous estimation of both drugs.

3.4.6. Robustness and Ruggedness

Robustness of the developed UV spectrophotometric method was evaluated by introducing small deliberate variations in the analytical conditions and observing their effect on absorbance. The study was performed by measuring the absorbance at 333 ± 2 nm for Thioctic acid and 298 ± 2 nm for Sesamol. The method showed consistent performance under the modified conditions, indicating good robustness.

Ruggedness of the method was assessed by analyzing the same sample under normal laboratory conditions by two different analysts. Absorbance values for concentrations of 2, 6, and 10 µg/mL were recorded and compared. The obtained percentage relative standard deviation (%RSD) values were found to be below 2%, confirming the repeatability, reproducibility, and reliability of the developed method. The detailed results of robustness and ruggedness studies are presented in Tables 5 and 6, respectively.

Table 5. Robustness with change in wavelength

Concentration (µg/ml)	% RSD	Change in Wavelength			
		Sesamol		Thioctic acid	
		296 nm	300 nm	331 nm	335 nm
2	1.24	0.654	0.456	0.214	
6	0.625	0.276	0.573	0.684	
10	0.251	0.254	0.475	0.451	

Table 6. Ruggedness with change in analyst

Concentration (µg/ml)	% RSD	Change in Analyst			
		Sesamol		Thioctic acid	
		Analyst 1	Analyst 2	Analyst 1	Analyst 2
2	0.452	0.954	0.745	0.147	
6	0.312	1.024	0.357	0.258	

10		0.475	0.456	0.954	0.546
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3.4.7. Repeatability

Sr. No.	Concentration (µg/ml)	Absorbance Sesamol	% RSD	Absorbance Thioctic acid	% RSD
1	8	0.488	0.59	0.461	0.42
2	8	0.486		0.464	
3	8	0.484		0.459	
4	8	0.487		0.459	
5	8	0.482		0.462	
6	8	0.490		0.461	

Repeatability of the developed UV spectrophotometric method was evaluated by performing repeated analysis of the same sample under identical experimental conditions within a short time interval. The obtained percentage relative standard deviation (%RSD) values were found to be less than 2%, indicating good repeatability and precision of the method. The detailed results are presented in Table 7.

Table 7

3.4.8. Forced Degradation Study

Forced degradation studies were carried out to evaluate the stability of Thioctic acid and Sesamol under different stress conditions, including acidic, alkaline, oxidative, and photolytic environments. Significant degradation was observed under oxidative and alkaline conditions, indicating the susceptibility of the drugs to these stress conditions. The study confirmed the stability-indicating capability of the developed UV spectrophotometric method. The obtained results are presented in Table 8.

Table 8: Results of forced degradation study

Forced Assay	Degradation	Degradation	
		Sesamol	Thioctic acid
Acidic		10.94%	12.23%
Basic		26.12%	1.24%
Oxidative		32.45%	13.5%
Sunlight		20.67%	100 %

3.4.9. Estimation of Thioctic Acid and Sesamol in Liposomal Formulation

With the growing application of vesicular drug delivery systems such as liposomes, the development of reliable and accurate analytical methods for formulation analysis is essential. Precise estimation of drugs incorporated within these vesicular formulations plays an important role in evaluating formulation quality, drug content, and therapeutic performance. In the present study, the validated UV spectrophotometric method was successfully applied for the simultaneous estimation of Thioctic acid and Sesamol in the developed liposomal formulations.

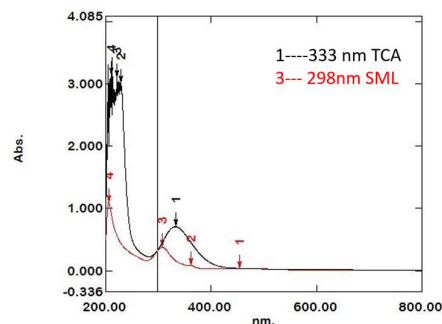


Figure 5. Estimation of Sesamol and Thioctic acid in liposomes

3.4.10. Assessment of Method Greenness and Whiteness Attributes:

The environmental sustainability of the developed UV spectrophotometric method for simultaneous estimation of Thioctic acid and Sesamol was evaluated using the Complex Green Analytical Procedure Index (ComplexGAPI). The generated pictogram demonstrated good environmental acceptability with predominant green and yellow zones, indicating reduced solvent consumption, lower chemical toxicity, and minimal waste generation. The central E-factor value further confirmed the eco-friendly nature of the developed analytical procedure.

The greenness profile of the developed method was further assessed using the Analytical GREENness (AGREE) metric, which evaluates compliance with the twelve principles of green analytical chemistry. The obtained AGREE score indicated that the method possesses good environmental compatibility due to its simple sample preparation, low solvent requirement, and reduced energy consumption. The corresponding AGREE pictogram displayed a predominantly green profile, confirming the environmentally favorable nature of the method. Method whiteness was additionally evaluated using the Blue Applicability Grade Index (BAGI), which integrates analytical performance, environmental sustainability, and practical applicability into a single assessment tool. The obtained BAGI score

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demonstrated that the developed UV spectrophotometric method achieved an appropriate balance between analytical efficiency, operational simplicity, and ecological safety, supporting its suitability for routine pharmaceutical analysis.

Overall, the greenness and whiteness assessments confirmed that the developed UV spectrophotometric method is environmentally sustainable, analytically reliable, and suitable for routine quality control analysis of Thioctic acid and Sesamol.

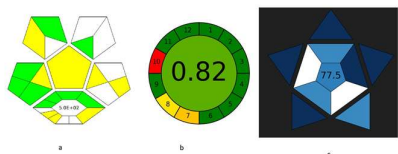


Figure 6. (a) GAPI pictogram illustrating the greenness assessment of the developed UV spectrophotometric method, (b) AGREE pictogram depicting the method's compliance with Green Analytical Chemistry principles, and (c) BAGI pictogram demonstrating the overall sustainability and greenness profile of the developed UV spectrophotometric method.

4. Conclusions

A simple, accurate, precise, and stability-indicating UV spectrophotometric method was successfully developed and validated for the simultaneous estimation of Thioctic acid and Sesamol. The developed method demonstrated good linearity, sensitivity, robustness, ruggedness, and repeatability in accordance with ICH Q2 (R1) guidelines. Forced degradation studies confirmed the stability-indicating capability of the method under different stress conditions.

The validated method was successfully applied for the estimation of Thioctic acid and Sesamol in liposomal formulations, indicating its suitability for vesicular drug delivery systems. In addition, greenness and whiteness assessment studies confirmed the environmentally sustainable and operationally efficient nature of the developed analytical procedure. Owing to its simplicity, cost-effectiveness, reliability, and eco-friendly profile, the developed UV spectrophotometric method can be effectively employed for routine quality control and pharmaceutical analysis.

Abbreviation

AGREE- Green Analytical Metric, BAGI- Blue applicability grade index, GAPI- Green Analytical Procedure Index, ICH- International council for harmonization, LOD- Limit of Detection, LOQ- Limit of Quantitation, TCA- Thioctic acid, SML- Sesamol.

Reference:

1. Sanmee S, Vipudhamorn W, Sutharat P, Supatrakul E. The efficacy of Aescin combined with MPFF for early control of bleeding from acute hemorrhoids, A randomized controlled trial. *Asian Journal of Surgery*. 2025 Jan 1;48(1):193-8.
2. Şahin F, Farshbaf-Khalili A, Alihosseini S, Sarbakhsh P, Pirouzpanah MS, Ayşan E, Doğan A, Gharekhani A, Khoshbaten M, Pirouzpanah MB. The efficacy of topical sodium pentaborate formulation on hemorrhoid disease: A randomized, double-blind, placebo-controlled trial. *Heliyon*. 2024 Mar 15;10(5).
3. Garrido JC, González GL. Effective non-surgical treatment of hemorrhoids with sclerosing foam and novel injection device. *Gastroenterology & Endoscopy*. 2024 Oct 1;2(4):176-80.
4. Pekacar S, Özüpek B, Akkol EK, Taştan H, Ersan H, Orhan DD. Identification of bioactive components on antihemorrhoidal activity of *Cistus laurifolius* L. using RP-HPLC and LC-QTOF-MS. *Journal of Ethnopharmacology*. 2024 Jan 30;319:117122.
5. Tanim TI, Al-Qaaneh AM, Chowdhury R, Bhuia MS, Islam T, Akbor MS, Islam MT, Miah MM, Ishaq AR, Abdel-Maksoud MA, El-Tayeb MA. Antiemetic activity of Sesamol possibly through serotonergic and dopaminergic receptor interaction pathways: In vivo and in silico studies. *Journal of Functional Foods*. 2025 Mar 1;126:106702.
6. Deol PK, Kaur IP, Dhiman R, Kaur H, Sharma G, Rishi P, Ghosh D. Investigating wound healing potential of sesamol loaded solid lipid nanoparticles: ex-vivo, in vitro and in-vivo proof of concept. *International Journal of Pharmaceutics*. 2024 Apr 10;654:123974.
7. Salehi B, Berkay Yılmaz Y, Antika G, Boyunegmez Tümer T, Fawzi Mahomoodally M, Lobine D, Akram M, Riaz M, Capanoglu E, Sharopov F, Martins N. Insights on the use of α -lipoic acid for therapeutic purposes. *Biomolecules*. 2019 Aug;9(8):356.
8. Optimization and Validation of a High-Performance Liquid Chromatography (HPLC- UV) Method for Quantification of α - Lipoic Acid in Cyclodextrins Complex for Ocular Delivery Phuong Linh Ta1 | Lucia Bernat-Just2 | Adrián M. Alambiaga-Caravaca2 | Vicent Rodilla2 | Alicia López-Castellano2 | Francisco Bosch-Morel
9. Šabanović M, Jašić M, Odobašić A, Aleksovska EŠ, Pavljašević S, Bajraktarević A, Čepo DV. Alpha lipoic

Development, Validation, and Greenness Assessment of a UV Spectrophotometric Method for Simultaneous Estimation of Thioctic Acid and Sesamol

- acid reduces symptoms and inflammation biomarkers in patients with chronic hemorrhoidal illness. *International journal for vitamin and nutrition research*. 2019 May 29.
10. Pawar A. Analytical Method Development and Validation for Venlafaxine Hydrochloride: UV-Visible Spectrophotometric Estimation in Bulk and Pharmaceutical Dosage Forms. *Journal of Internal Medicine and Pharmacology (JIMP)*. 2025 Jul 20;2(03):01-13.
 11. Ibrahim MA, Algohary AM, Al-Ghamdi YO, Ibrahim AM. A Green analytical method for simultaneous determination of dexamethasone sodium phosphate and prednisolone acetate in veterinary formulations using UV spectroscopy and dimension reduction algorithms. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2025 Mar 5;328:125446.
 12. Akabari AH, Solanki DK, Patel SK, Desai P, Jainisha G, Patel B, Shah BN, Shah KV. Development and validation of a novel simultaneous equation and Q-absorbance ratio method for the quantitative estimation of atenolol and hydrochlorothiazide in combined tablet dosage forms: a green analytical chemistry approach. *Green Analytical Chemistry*. 2025 Mar 1;12:100224.
 13. Prete P, Iannaccone D, Proto A, Tobiszewski M, Cucciniello R. Development and validation of an eco-compatible UV-Vis spectrophotometric method for the determination of Cu²⁺ in aqueous matrices. *Analytical and Bioanalytical Chemistry*. 2023 Aug;415(20):5003-10.
 14. Chauhan I, Singh LU. Development and validation of a simple and cost-effective UV spectrophotometric method for quantifying linezolid. *Int J App Pharm*. 2024;16(3):211-6.
 15. Evalina TR, Azzam M, Sari M, Gadhav A, Septiana L. Validated Mean Centering Ratio UV Spectrophotometric Method for the Determination of Bromhexine Hydrochloride and Guaifenesin in Syrup Dosage Form. *Asian Journal of Pharmaceutical Research and Development*. 2026 Feb 15;14(01):1-5.
 16. Blessy MR, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. *J Pharm Anal*. 2014;4(3):159–165.
 17. International Conference on Harmonisation (ICH). Stability Testing of New Drug Substances and Products Q1A(R2). Geneva: ICH; 2003.
 18. Biscaia-Caleiras M, Fiteiro J, Lopes D, Fidalgo T, Lourenço AS, Moreira JN, Simões S. Implementation of design of experiments in liposome manufacturing: ethanol injection and extrusion as a case study. *Journal of Drug Delivery Science and Technology*. 2025 Oct 1;112:107236.
 19. Yenduri S, Varalakshmi HN. Assessment and comparison of sustainability aspects of UV-spectroscopy methods for simultaneous determination of anti-hypertensive combination. *Green Analytical Chemistry*. 2024 Jun 1;9:100108.
 20. Darwish AS, Gouda AA, El Sheikh R, Alenezia SS, Ghaith ES. Gradient mode HPLC approach to quantify sodium bisulfite as an antioxidant in raw material and in oral pharmaceutical suspension containing parabens and nystatin; utilization of greenness tools. *Results in Chemistry*. 2025 Dec 16:102985.
 21. Ma JK, Chen XY, Zhang N, Darwish AS, Gouda AA, El Sheikh R, Huang XC. A straightforward HPLC approach to testing butylated hydroxytoluene, an antioxidant, in pure and topical anti-burn gels; evaluation of greenness, blueness, and whiteness grades. *Talanta Open*. 2025 Sep 23:100565.
 22. Huang XC, Darwish AS, Darwish WS, Chen RM, Ma JK. Green rapid HPLC method for testing retinol and tocopherol in ophthalmic gels. *Talanta Open*. 2025 Aug 28:100538.

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Use of Artificial Intelligence (AI)-Assisted Technology

The authors confirm that artificial intelligence (AI)-assisted tools were not used in the preparation, writing, or editing of this manuscript.