Development and Validation of a UV Spectrophotometric Method for the Estimation of Syringic Acid and Its Application in NLC-Based Nano-formulation

Priya Helavi[1], Nisha Shirkoli[1*]

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

Corresponding author: Mrs. Nisha Shirkoli | Assistant Professor, Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy Belagavi- 591124, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, India | Email: nishashirkoli@klepharm.edu

ORCID IDs: Nisha Shirkoli: 0000-0003-4927-8794 | Priya Helavi: 0009-0008-2010-2107

ABSTRACT

The present study aimed to develop and validate a stability-indicating UV spectrophotometric method for the estimation of Syringic acid and to apply the method in NLC nanoformulations using water as a green solvent. A 3^2 full factorial design was employed to investigate the influence of sonication time and scanning interval on absorbance, while ANOVA-based optimization helped establish a robust analytical design space. The developed method demonstrated excellent linearity over the concentration range of 2–10 µg/mL, with satisfactory sensitivity, robustness, and ruggedness in accordance with International Council for Harmonisation Q2 (R2) guidelines. Forced degradation studies under acidic, alkaline, oxidative, photolytic, and thermal stress conditions showed distinct spectral separation between the intact drug and its degradation products, thereby confirming the stability-indicating nature of the method. In addition, the validated method was successfully applied for the estimation of the drug in NLC formulations, proving its suitability for routine quality control and nanoformulation analysis.

Keywords: Syringic acid, UV spectrophotometry, NLCs, Nanoformulation.

How to cite this article: Helavi P, Shirkoli N. Development and Validation of a UV Spectrophotometric Method for the Estimation of Syringic Acid and Its Application in NLC-Based Nano-formulation. Int J Drug Deliv Technol. 2026;16(54s): 950-958. DOI: 10.25258/ijddt.16.54s.82

Source of support: Nil.

Conflict of interest: None.

1. Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor, accounting for about 16% of all brain and central nervous system tumors, with an incidence rate of 3.2 cases per 100,000 people. It mainly occurs in the brain but may also affect the brain stem, cerebellum, and spinal cord. GBM is more common in older adults, especially men, and is slightly more prevalent in Caucasians (1). It is classified as primary GBM, which develops suddenly, or secondary GBM, which progresses from lower-grade tumors. Most cases are primary and are associated with a poorer prognosis. Although patients may initially respond to treatment, tumor recurrence is very common. Treatment options for recurrent GBM include surgery, radiotherapy, chemotherapy, bevacizumab, and tyrosine kinase inhibitors (2). The median survival period for most patients remains approximately 12 to 18 months, while the long-term survival rate is still very low (3). Recent developments in molecular biology and genomic profiling have greatly enhanced the understanding of Glioblastoma pathogenesis and have led to major changes in the diagnostic classification of central nervous system tumors (4).

Syringic acid (SA) is a naturally occurring phenolic acid found in several plants and food sources such as walnuts, black olives, cinnamon, and sesame. Clinically, SA has been recognized for its broad-spectrum antimicrobial and strong antioxidant properties (6). Recent pharmacological research has shown that SA exhibits multiple biological activities, including antitumor effects, chemopreventive action against skin cancer, and antithrombotic properties. Preliminary studies have also indicated that this phenolic compound demonstrates significant anti-proliferative activity against human colorectal and breast cancer cells (7). Due to its diverse biological properties and natural availability, Syringic acid has gained increasing interest in pharmaceutical, nutraceutical, and biomedical research in recent years. Syringic acid exhibits strong antioxidant activity by neutralizing free radicals and enhancing the function of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (8).

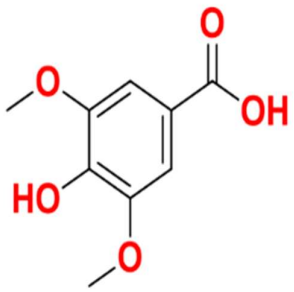

Figure 1: Chemical Structure of syringic acid

In recent years, several analytical techniques have been reported for the qualitative and quantitative determination of Syringic acid in different matrices such as pure drug samples, herbal formulations, and biological systems. These methods include ultraviolet–visible (UV–Vis) spectrophotometry (9), ultra-performance liquid chromatography (UPLC) (10), and advanced hyphenated techniques like fluorescence liquid chromatography–mass spectrometry (FLC–MS/MS) (11) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (12, 14). The development of these analytical approaches reflects the increasing demand for accurate, sensitive, and high-throughput methods for the estimation of syringic acid in complex phytochemical and biological matrices.

2. Methodology

2.1. Materials:

Syringic acid (SYN, purity > 99%) was procured from Yarrow Chemicals Pvt. Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade and procured from KLE College of Pharmacy, Belagavi, India.

2.2. Instrumentation:

All spectrophotometric analyses were carried out using a Shimadzu UV-1900i Spectrophotometer integrated with Lab Solutions software. Method optimization and experimental design were conducted using Design-Expert software (Stat-Ease Inc., Minneapolis, MN, USA). The experiments were performed at ambient room temperature, with double-distilled water employed as the blank solvent throughout the study.

2.3. Selection of Solvent

Various analytical solvents such as water, ethanol, methanol, and dimethyl sulfoxide (DMSO) were investigated during the initial experimental studies. Considering the solubility profile and quality of the obtained spectra, water was chosen as the most suitable solvent for the development of the analytical method.

2.4. Preparation of Stock Solution:

Accurately weighed 10 mg of Syringic acid was dissolved in methanol to prepare the primary stock solution. Subsequently, 1 mL of this solution was transferred and diluted to 10 mL with methanol to obtain the secondary stock solution. Further appropriate dilutions were carried out using water to prepare working standard solutions in the concentration range of 2–10 µg/mL, followed by sonication for 5 minutes.

2.5. Selection of Wavelength

Syringic acid was analysed over the wavelength range of 200–400 nm using UV spectrophotometry to identify its maximum absorbance wavelength (λmax). The compound exhibited a characteristic absorption maximum at 272 nm.

2.6. Method Validation

The developed method was validated according to ICH Q2 (R1) guidelines, assessing specificity, linearity, precision and accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness, and repeatability(15).

2.6.1. Specificity and Selectivity

Specificity of the method was evaluated by examining the potential interference of formulation excipients. The blank samples showed no significant absorbance at 272 nm, confirming the selective and interference-free estimation of Syringic acid.

2.6.2. Linearity

Linearity of the method was evaluated over the concentration range of 2–10 µg/mL by constructing calibration curves between absorbance and concentration. The regression equation and correlation coefficient (R^2) were determined to verify the linear relationship of the method.

2.6.3. Precision

Precision of the method was determined through repeatability, intraday precision, and interday precision studies. Three different concentrations of Syringic acid were analyzed in triplicate. Intraday precision was assessed on the same day, whereas interday precision was evaluated over three successive days. The percentage relative standard deviation (%RSD) was calculated to establish the reproducibility and consistency of the method.

2.6.4. Accuracy

Accuracy of the method was determined through recovery studies performed at 80%, 100%, and 120% of the target concentration. The percentage recovery was calculated to evaluate the agreement between the measured values and the actual amount of Syringic acid present in the sample.

2.6.5. Sensitivity (LOD and LOQ)

Sensitivity was determined using the limit of detection (LOD) and limit of quantification (LOQ), calculated based on signal-to-noise ratios of approximately 3:1 and 10:1, respectively.

2.6.6. Ruggedness

Ruggedness of the method was evaluated by conducting the assay using different analysts and instruments under varying laboratory conditions. The percentage relative standard deviation (%RSD) was determined to verify the reproducibility and reliability of the method.

2.6.7. Robustness

Robustness of the method was assessed by intentionally introducing small variations in the analytical conditions, including a ±2 nm change from the λmax. The percentage relative standard deviation (%RSD) was calculated to evaluate the ability of the method to remain unaffected by minor procedural modifications.

2.6.8. Repeatability

Repeatability of the method was evaluated by recording the absorbance of Syringic acid six times at a concentration of 6 µg/mL. The percentage relative standard deviation (%RSD) was calculated to verify the consistency and precision of the method under identical operating conditions.

2.7. Forced Degradation Study

Forced degradation studies were performed to establish the stability-indicating capability of the method. The drug was subjected to various 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% H_2O_2 at 80°C for 2 h), and thermal stress (40°C for 4 h). Following treatment, the solutions were diluted with water to obtain a concentration of 10 µg/mL and scanned over the wavelength range of 200–400 nm. Significant degradation was observed under the applied stress conditions, demonstrating clear spectral differentiation between the degraded and undegraded forms of Syringic acid(16,17).

2.8. Preparation of Nanostructured lipid carriers

Syringic acid-loaded nanostructured lipid carriers (SYN-NLCs) were prepared using hot homogenization followed by ultrasonication. SYN was dissolved in stearic acid (1.2% w/v) and heated at 60 °C with continuous stirring at 600 rpm. Separately, Labrafil M 2130 CS (0.3% w/v) and Tween 80 (0.5% w/v) were heated and mixed under similar conditions. The lipid phase was slowly added to the drug-containing phase, followed by dropwise addition of Millipore water to obtain a final volume of 30 mL. The dispersion was homogenized at 10,000 rpm for 10 minutes and then probe-sonicated for 10 minutes with a 10-second pulse interval. The prepared SYN-NLCs were stored at room temperature for further characterization studies(18,19).

2.9. Application of the Method to Nano formulation

The validated UV spectrophotometric method was successfully employed for the estimation of Syringic acid in NLC formulations. An accurately measured quantity of the formulation was dispersed in water, sonicated, suitably diluted, and analyzed spectrophotometrically at its respective λmax value.

2.10. Greenness Assessment of the Developed Method:

The developed UV spectrophotometric method for Syringic acid was evaluated for its environmental sustainability using Complex GAPI, AGREE, and BAGI assessment tools. The integrated analysis indicated that the method is eco-friendly, analytically efficient, and suitable for routine practical applications.

Complex GAPI was employed to assess the environmental impact associated with each stage of the analytical procedure, including sample preparation, solvent usage, instrumentation, energy requirements, and waste production. The dominance of green and light-yellow regions in the assessment reflected minimal sample processing, low solvent toxicity, and lower energy consumption, thereby confirming the eco-friendly nature of the developed method(20).

The Analytical Greenness (AGREE) metric was used to quantitatively evaluate the compliance of the developed method with the twelve principles of Green Analytical Chemistry. AGREE generates a normalized greenness score ranging from 0 to 1 along with a circular graphical representation for easy interpretation. The assessment of the developed UV spectrophotometric method demonstrated its environmentally friendly nature, attributed to low solvent usage, reduced energy consumption, and the use of simple analytical instrumentation(21).

Furthermore, the Blue Applicability Grade Index (BAGI) was utilized to provide a comprehensive evaluation by integrating green chemistry, sustainability, and analytical applicability into a single assessment system. In addition to environmental considerations such as solvent safety and waste production, BAGI also evaluates analytical efficiency, ease of operation, energy consumption, and user safety. The developed method achieved a high BAGI score, indicating excellent methodological “whiteness” and demonstrating a well-balanced combination of sustainability and analytical reliability. Overall, the greenness assessment confirmed that the proposed UV spectrophotometric method is eco-friendly, dependable, and appropriate for the routine estimation of Syringic acid in nanoformulation-based drug delivery systems(22).

3. Results and Discussion

3.1. Selection of Solvent and Wavelength

Among the various solvents investigated, water was identified as the most suitable solvent based on its excellent solubility profile and spectral clarity. Consequently, all experimental studies were carried out using water as the blank solvent. Syringic acid was scanned over the wavelength range of 200–400

nm at a concentration of 10 µg/mL, and the λmax was found to be 272 nm.

3.2. Method Validation

Validation of analytical methods is important to ensure regulatory compliance, adherence to scientific requirements, and maintenance of quality control standards. The optimized method was comprehensively validated in accordance with International Council for Harmonisation Q2 (R1) guidelines.

3.2.1. Specificity and Selectivity

Selectivity of an analytical method refers to its ability to accurately determine the analyte in the presence of possible interfering substances within the sample matrix. The specificity of the developed method was confirmed by the characteristic maximum absorbance of Syringic acid at 272 nm. Furthermore, the absence of absorbance at this wavelength in the solvent spectrum established the selective nature of the method.

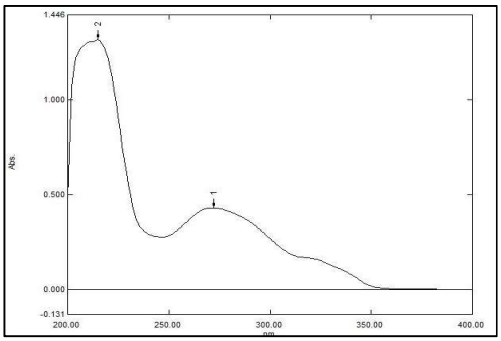

Figure.2. UV spectrum of syringic acid

The UV spectrum illustrated in Figure 2 showed that syringic acid exhibited maximum absorbance (λmax) at 272 nm. The observed wavelength was found to be in agreement with previously reported literature values.

3.2.2. Linearity

Method linearity refers to the ability of an analytical procedure to produce results that are directly proportional to the concentration of the analyte within a defined range. A strong linear relationship between concentration and absorbance was observed for Syringic acid over the concentration range of 2–10 µg/mL, with a regression coefficient (R^2) of 0.997. Figures 2C illustrate the calibration curve of syringic acid at 272 nm, demonstrating excellent linearity between concentration and absorbance within the studied range.

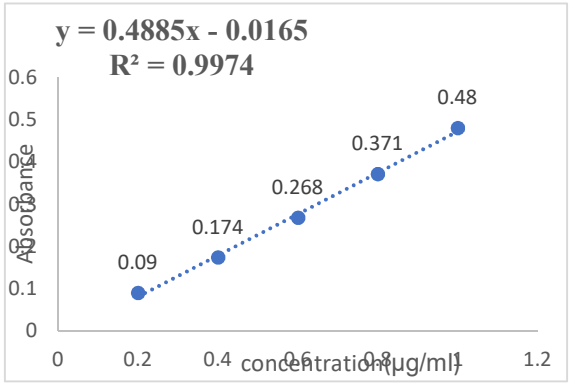

Figure.3. Standard Calibration Curve of syringic acid

Table 1. Linearity of Syringic acid.

sr. no.	Concentration (µg/ mL)	Absorbance of S Syringic acid (272)
1	0	0.000
2	2	0.09
3	4	0.174
4	6	0.268
5	8	0.371
6	10	0.48
R^2		0.997
LOD		0.2 µg/mL
LOQ		0.6 µg/mL

3.2.3. System Precision

The precision of the method was established through both intraday and interday studies. Intraday precision was determined by measuring absorbance at three different time intervals morning, afternoon, and evening on the same day, whereas interday precision was evaluated over three consecutive days. The developed method demonstrated good precision, with all percentage relative standard deviation (%RSD) values found to be below 2%. Detailed results are presented in Tables 2 and 3.

Table 2. Intraday precision assay

Syringic acid precision (n=3)				
Concentration (µg/ml)	% RSD	Morning	Afternoon	Evening
2	1.12	0.092	0.0.089	0.095
6	0.37	0.268	0.270	0.275
10	0.21	0.481	0.483	0.486

Table 3. Interday precision assay

Syringic acid Interday precision (n=3)				
Concentration (µg/ml)	%RSD	Day 1	Day 2	Day 3
2	1.11	0.091	0.090	0.089
6	0.37	0.269	0.267	0.268
10	0.32	0.481	0.480	0.478

3.2.4. Accuracy

Accuracy represents the degree of agreement between the experimental value and the true value of the analyte. The recovery percentage of Syringic acid was observed to be in the range of 96.8% to 101.6%. The uniform recovery results obtained at different concentration levels demonstrated the effectiveness and acceptable accuracy of the developed analytical method. Detailed results are presented in Table 4.

Table 4. Accuracy/ percentage recovery

Sr. No.	Concentration (µg/mL)	Absorbance	%RSD
1	6	0.268	0.51
2	6	0.267	
3	6	0.269	
4	6	0.266	
5	6	0.270	
6	6	0.268	

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 calculated using 10 times the same ratio. For Syringic acid, the LOD and LOQ values were found to be 0.2 µg/mL and 0.6 µg/mL, respectively. These results indicate the excellent sensitivity and reliability of the proposed method.

3.2.6. Robustness and Ruggedness

Robustness of an analytical method reflects its ability to remain unaffected by small, intentional variations in experimental conditions, thereby demonstrating the reliability and consistency of the method during routine laboratory analysis. The robustness of the developed method was evaluated by recording the absorbance of Syringic acid at 272 ± 2 nm. Ruggedness represents the reproducibility of the method under different normal operating conditions. It was assessed by two different analysts using concentrations of 2, 6, and 10 µg/mL. The low percentage relative standard deviation (%RSD) values, all below 2%, confirmed the repeatability, reproducibility, robustness, and ruggedness of the developed analytical method. Detailed results are presented in Tables 5&6.

Forced Degradation Condition	Degradation (%)
Acidic	12.94%
Alkaline	26.12%
Oxidative	100%
Photolytic (Sunlight)	25.67%

Table 5. Ruggedness with change in analyst

Concentration (µg/mL)	Analyst 1 (%RSD)	Analyst 2 (%RSD)
2	0.090	0.092
6	0.268	0.271
10	0.480	0.482

Table 6. Robustness with change in wavelength

LEVEL	Concentration (µg/ml)	Absorbance (272 nm)	% Recovery
80%	4	0.176	101.2%
		0.174	
		0.175	
100%	6	0.269	99.8%
		0.267	
		0.268	
120%	8	0.373	98.7%
		0.370	
		0.371	

3.2.7. Repeatability

Intra-assay precision, commonly referred to as repeatability, represents the capability of an analytical method to produce consistent results within a short duration under identical experimental conditions. A percentage relative standard deviation (%RSD) value below 2% indicates that the developed method possesses good repeatability and precision. Detailed results are presented in Table 7.

Table 7: Repeatability

3.2.8. Forced degradation study

Forced degradation studies revealed that Syringic acid was susceptible to acidic, alkaline, oxidative, and photolytic stress conditions. Significant degradation was particularly observed under

oxidative and alkaline environments, thereby confirming the stability-indicating capability of the developed analytical method. Detailed results are presented in Table 8.

Table 8: Results of forced degradation study

Concentration (µg/mL)	270 nm (%RSD)	272 nm (%RSD)
2	0.085	0.096
6	0.263	0.269
10	0.472	0.479

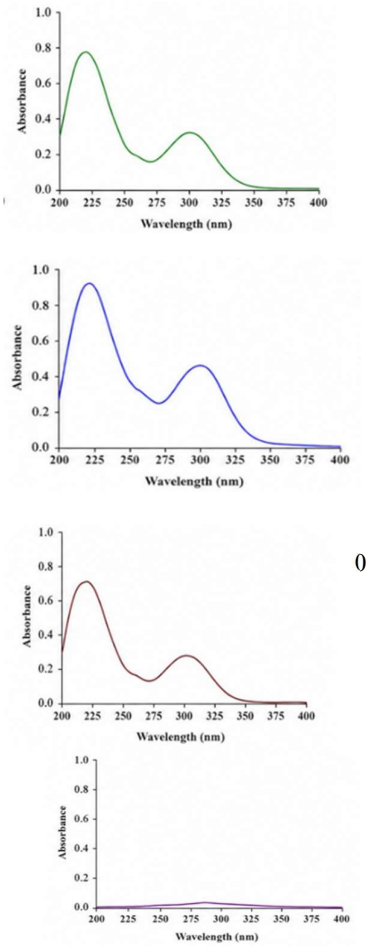

Figure. 4. a) Acidic b) Basic c) oxidative d) photolytic , Forced Degradation assay of Syringic acid.

3.2.9. Estimation of syringic acid for NLCs formulation

With the growing development of nanocarrier-based drug delivery systems, the need for reliable and accurate analytical methods for quality evaluation has become increasingly important. Precise estimation of drugs incorporated within nanoformulations is essential, as it significantly influences therapeutic effectiveness and formulation performance. In the present study, a validated UV spectrophotometric method was successfully developed and applied for the estimation of Syringic acid in NLC formulations. Detailed results are presented in Table 9.

Table 9. Estimation of Syringic acid from nanoformulation.

Formulation	Particle Size(nm)	PDI	Zeta Potential(mV)
Syringic acid loaded NLCs	179.3	0.12	-26.16

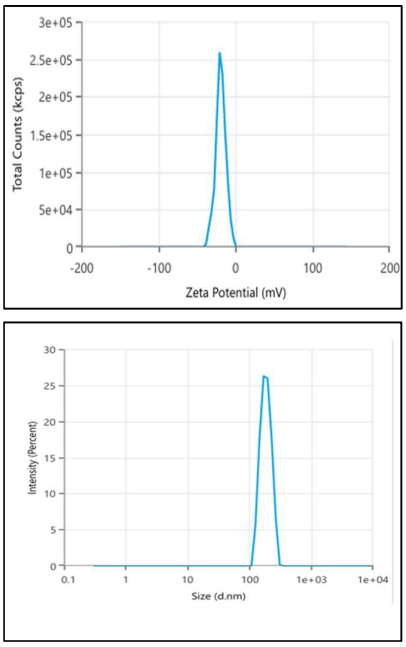

Figure. 5 . Particle size (a) & Zeta Potential (b) of the Optimized formulation

Assessment of Method Greenness and Whiteness Attributes:

The environmental sustainability of the developed UV spectrophotometric method for Syringic acid was evaluated using the Complementary Green Analytical Procedure Index (ComplexGAPI). The obtained pictogram contained seven green, nine yellow, and one red section, suggesting that the method is environmentally acceptable. The green areas represented the use of low-toxicity solvents and minimal solvent consumption, whereas the yellow sections were mainly related to sample preparation procedures and instrument energy requirements. Additionally, the central E-factor value of 1.0 indicated low waste production, confirming that the developed method complies well with the principles of green analytical chemistry.

The environmental compatibility of the developed UV spectrophotometric method was further evaluated using the Analytical GREEnness (AGREE) metric, which quantitatively assesses compliance with the twelve principles of green analytical chemistry. The developed method

achieved a high AGREE score of 0.8, and the associated pictogram exhibited a predominantly green appearance, indicating excellent environmental sustainability and minimal ecological impact.

The whiteness of the developed method was further assessed using the Blue Applicability Grade Index (BAGI), which integrates analytical efficiency, environmental sustainability, and practical applicability into a unified evaluation system. The method achieved a BAGI score of 70, indicating a well-balanced analytical procedure with good robustness and environmental acceptability. These findings confirmed the suitability of the developed UV spectrophotometric method for the routine analysis of Syringic acid.

Overall, the greenness and blueness evaluations demonstrated that the developed UV spectrophotometric method effectively combines analytical reliability with environmental sustainability and practical efficiency. The method showed low solvent consumption, minimal waste production, and good robustness, making it highly suitable for routine high-throughput analysis in pharmaceutical quality control and regulatory laboratories.

(a) (b) (c)

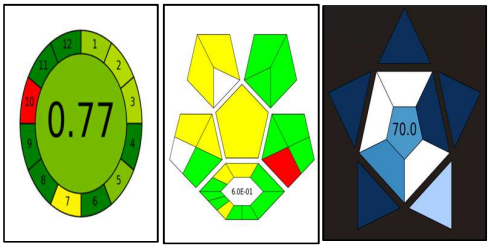

Figure. 8. a) GAPI pictogram representing the greenness profile of the developed UV spectrophotometric method, b) AGREE pictogram representing the green analytical profile of the developed UV spectrophotometric method, c) BAGI pictogram representing the greenness profile of the developed UV spectrophotometric method.

3.Conclusions

The present study successfully developed and validated a simple, accurate, robust, and stability-indicating UV spectrophotometric method for the estimation of Syringic acid in NLC nanoformulations. Water was selected as the analytical solvent due to its excellent solubility and spectral clarity. The drug showed a maximum absorbance (λmax) at 272 nm. The method exhibited excellent linearity in the concentration range of 2–10 µg/mL with an R^2 value of 0.997. Validation was performed according to International Council for Harmonisation Q2 (R2) guidelines. The method demonstrated good specificity, precision, accuracy, robustness, ruggedness, and repeatability. Precision studies showed %RSD values below 2%, indicating good reproducibility. Recovery studies confirmed satisfactory accuracy, with recovery values ranging from 96.8% to 101.6%. The LOD and LOQ values were found to be 0.248 µg/mL and 0.651 µg/mL, respectively, indicating high sensitivity. Robustness and ruggedness studies confirmed the reliability of the method under varied analytical conditions. Overall, the developed UV method was found to be reliable, eco-friendly, and suitable for routine analysis of syringic acid in NLC formulations.

Acknowledgments

We express our sincere gratitude to the Principal of KLE College of Pharmacy for providing the essential facilities and continuous support required for carrying out this research work. Their encouragement, guidance, and valuable assistance greatly contributed to the successful completion of the study.

Author contributions

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

Author 3- Formal analysis, Validation, Writing- Review & editing.

Financial support

The authors declare that they have no financial relationships or conflicts of interest related to the subject matter or materials presented in this manuscript.

Disclosure of conflicts

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

Use of artificial intelligence (AI)-assisted technology

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

References

1. Davis ME. Glioblastoma: overview of disease and treatment. Clinical journal of oncology nursing. 2016 Oct 1;20(5): S2.

2. Shergalis A, Bankhead III A, Luesakul U, Muangsin N, Neamati N. Current challenges and opportunities in treating glioblastoma. Pharmacological reviews. 2018 Jul 1;70(3):412-45.

3. Ostrom QT, Patil N, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United

States in 2013–2017. Neuro-oncology. 2020 Oct;22(Supplement_1):iv1-96.

4. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro-oncology. 2021 Aug 1;23(8):1231-51

5. Liu Y, Sun C, Li W, Adu-Frimpong M, Wang Q, Yu J, Xu X. Preparation and characterization of Syringic acid–loaded TPGS liposome with enhanced oral bioavailability and in vivo antioxidant efficiency. Aaps Pharmscitech. 2019 Apr; 20:1-0.

6. Orabi KY, Abaza MS, El Sayed KA, Elnagar AY, Al-Attiyah R, Guleri RP. Selective growth inhibition of human malignant melanoma cells by syringic acid-derived proteasome inhibitors. Cancer cell international. 2013 Dec; 13:1-6.

7. Bartel I, Mandryk I, Horbańczuk JO, Wierzbicka A, Koszarska M. Nutraceutical properties of syringic acid in civilization diseases. Nutrients. 2023 Dec 19;16(1):10.

8. Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Kumar CS. Syringic acid (SA)–a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomedicine & Pharmacotherapy. 2018 Dec 1;108:547-57.

9. Leow SS, Sambanthamurthi R, Mohd Hassim NA, Ismail NH, Saw MH, Wazir H, Tang TK, Kanagaratnam S. Gradient UPLC-UV/PDA method validation for simultaneous quantification of major phenolic acid compounds in water-soluble palm fruit extract (WSPFE). Food Analytical Methods. 2025 Nov;18(11):2457-69.

10. Barbaro E, Feltracco M, Spagnesi A, Dallo F, Gabrieli J, De Blasi F, Zannoni D, Cairns WR, Gambaro A, Barbante C. Fast Liquid Chromatography coupled with tandem mass spectrometry for the analysis of vanillic and syringic acids in ice cores. Analytical chemistry. 2022 Mar 23;94(13):5344-51.

11. Hernanz D, Jara-Palacios MJ, Santos JL, Pajuelo AG, Heredia FJ, Terrab A. The profile of phenolic compounds by HPLC-MS in Spanish oak (Quercus) honeydew honey and their relationships with color and antioxidant activity. LWT. 2023 Apr 15; 180:114724.

12. Wang Y, Xing L, Zhang J, Ma X, Weng R. Determination of endogenous phenolic compounds in honey by HPLC-MS/MS. LWT. 2023 Jun 15; 183:114951.

13. Paganelli CJ, Siebert DA, Vitali L, Micke GA, Alberton MD. Quantitative analysis of phenolic compounds in crude extracts of Myrcia splendens leaves by HPLC-ESI-MS/MS. Rodriguésia. 2020 Jul 13;71: e00552019.

14. Li F, Tsona NT, Li J, Du L. Aqueous-phase oxidation of syringic acid emitted from biomass burning: Formation of light-absorbing compounds. Science of The Total Environment. 2021 Apr 15; 765:144239.

15. Ermer J. ICH Q2(R2): Validation of analytical procedures. In: *Method Validation in Pharmaceutical Analysis: A Guide to Best Practice.* Weinheim: Wiley-VCH; 2025. p. 351–372.

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. Mittal S, Shah S, Yadav HN, Ali J, Gupta MM, Baboota S. Quality by design engineered, enhanced anticancer activity of temozolomide and resveratrol coloaded NLC and brain targeting via lactoferrin conjugation in treatment of glioblastoma. European Journal of Pharmaceutics and Biopharmaceutics. 2023 Oct 1;191:175-88.

19. Barman M, Gaur PK, Nagpal D. Formulation and Evaluation of Nano-particles Loaded with Salvia miltiorriza by Homogenization Method. Nanoscience & Nanotechnology-Asia. 2024 Dec;14(6):E22106812312265.

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.

Figure Captions

Table Captions