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Development and Validation of a Stability-Indicating RP-HPLC Method for the Quantification of Embelin in Pure Form and Transethosomal Formulations

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Abstract

This research outlines the development and validation of a reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative analysis of embelin, a biologically active hydroxyquinone predominantly sourced from *Embelia ribes*. Recognized for its diverse pharmacological properties including antioxidant, antidiabetic, anti-inflammatory, and anticancer activities embelin was analyzed using a Phenomenex Luna C18(2) column with a mobile phase composed of acetonitrile and 0.1% orthophosphoric acid (90:10 v/v), at a flow rate of 1.0 mL/min, with detection at 291 nm. The method was validated according to ICH Q2(R1) guidelines, assessing key parameters such as linearity, accuracy, precision, robustness, and the limits of detection (LOD) and quantification (LOQ). The calibration curve displayed strong linearity (R^2 = 0.9988) within the concentration range of 2–10 µg/mL. Sensitivity was confirmed with an LOD of 0.111 µg/mL and an LOQ of 0.555 µg/mL. Stability-indicating capability was demonstrated through forced degradation studies under various stress conditions, including acidic, basic, thermal, oxidative, and photolytic environments. The validated method was effectively applied to determine embelin content in transethosomal formulations, confirming its suitability for routine pharmaceutical quality control and analytical applications.

Key words: Embelin, Transethosomes, Analytical method validation, RP-HPLC

1. INTRODUCTION

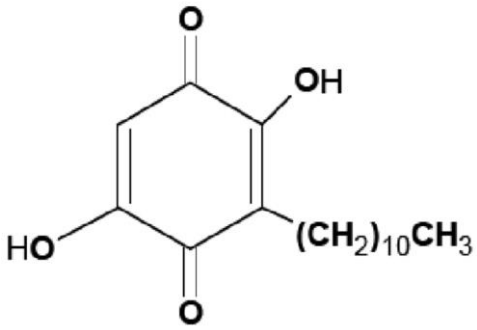
Embelin(2,5-dihydroxy-3-undecyl-1,4-benzoquinone) is an alkyl-substituted hydroxyquinone primarily isolated from Embelia ribes and several other medicinal plants. It exhibits a broad spectrum of biological activities and is often responsible for the traditional medicinal use of its natural sources. Extensive studies have demonstrated its diverse pharmacological properties, highlighting its potential applications in clinical research.This review provides a comprehensive overview of existing literature on embelin, with a particular focus on its antidiabetic, cardioprotective, neuroprotective, anti-inflammatory, antiviral, antimicrobial, antioxidant, wound healing, antifertility, analgesic, and anticancer activities.Moreover, the review emphasizes the chemical characteristics of embelin and explores environmentally sustainable

methods for its large-scale production. Continued research is essential to fully exploit its therapeutic potential and contribute to the development of novel phyto-pharmaceuticals based on this natural compound[1]. In recent decades, there has been a significant increase in research focused on natural antioxidants, largely due to their non-toxic nature and their ability to interact with various endogenous and exogenous free radicals within living cell [2]. This study investigates the interactions of embelin with oxidizing free radicals and evaluates its antioxidant activity in various in vitro systems. Among physiologically relevant radicals, the peroxyl radical is known to cause oxidative damage to critical biological molecules such as lipids, proteins, and DNA [3]. The protective role of embelin against such oxidative damage has been demonstrated *in vitro* using rat liver mitochondria. Specifically, the effects of embelin on hydroxyl radical-induced deoxyribose degradation and peroxyl radical-induced loss of mitochondrial manganese superoxide dismutase (Mn-SOD) activity have been examined. Additionally, embelin's redox potential has been assessed through multiple assays, including total phenolic content estimation, Fe(III) reduction, and cyclic voltammetry. To further elucidate the molecular mechanisms underlying its free radical scavenging activity, nanosecond pulse radiolysis techniques have been employed [4].

Melasma is a common acquired skin disorder characterized by symmetric hyperpigmented macules or patches, typically appearing on sun-exposed areas of the skin most notably the face, but also potentially affecting the neck and forearms. It is often referred to as the "mask of pregnancy" due to its frequent occurrence during pregnancy or in individuals using hormonal contraceptives. Although melasma is a benign condition and may resolve spontaneously, particularly when hormonal triggers are removed, various treatment options are available to reduce pigmentation and improve skin appearance [5]. Many women first notice blotchy patches and freckle-like spots on their face during pregnancy or after beginning hormonal contraceptives, such as birth control pills. Due to its high prevalence during pregnancy, melasma is often referred to as the “mask of pregnancy.” In some cases, the condition resolves on its own after childbirth or upon discontinuation of hormonal contraceptives [6]. Melasma is more commonly seen in women with medium to dark skin tones, who are more prone to developing hyperpigmentation. When it occurs, melasma typically presents as tan, brown, grayish-brown, or bluish-gray patches and freckle-like spots. These discolorations most often appear on the face particularly the cheeks, forehead, chin, and the area above the upper lip. Although less common, melasma can also affect other sun-exposed areas such as the neck, arms, or forearms[7]. Topical treatments, such as prescription creams containing hydroquinone, tretinoin, or corticosteroids, can help lighten melasma and reduce discoloration. However, these treatments do not offer a permanent cure, as melasma often recurs [8]. Sun exposure is a major trigger, and it is common for melasma to return if the skin is not adequately protected. Consistent use of broad-spectrum sunscreen and protective clothing is essential to help prevent flare-ups and maintain treatment results [9] In fact, many individuals with melasma report that the dark spots and patches become more pronounced during the summer months and tend to fade during the winter. This seasonal variation is largely due to increased sun exposure. As a result, daily sun protection is essential The separation of sample components is achieved based on their varying interactions with the stationary phase, allowing for precise and efficient analysis of complex mixtures.sunscreen with high SPF and wearing a wide-brimmed hat can help prevent the darkening of existing patches and reduce the likelihood of recurrence [10].

Applying a broad-spectrum High-Performance Liquid Chromatography (HPLC**)** is a powerful analytical technique used for the separation, identification, and quantification of components within a mixture. It is a form of column chromatography in which a liquid sample is forced through a column packed with a stationary phase under high pressure, using a liquid mobile phase [11]. Embelin can be effectively analyzed using High-Performance Liquid Chromatography (HPLC), a technique that separates and quantifies components in a sample based on their differential interactions with a stationary phase. HPLC is widely employed for the quantification of embelin in various contexts, including pharmaceutical analysis, quality control of herbal formulations, and phytochemical investigations. Its high sensitivity, accuracy, and reproducibility make HPLC a preferred method for embelin analysis [12]. The embelin content was determined using the HPLC method described by Choudhary et al. with some modifications to optimize the analysis [13].

The objective of this investigation was to develop a chromatographic analysis method that is both simple and accurate for the detection and quantification of embelin in its pure form as well as in pharmaceutical formulations. Previous analytical methods, although widely used, often require complex instrumentation and lengthy analysis times, which can limit their practicality for routine applications**[**14].



(2,5-dihydroxy-3-undecyl-1,4-benzoquinone)

Figure 1 Structure of Embelin

Materials and Methods

Chemicals and reagent

Embelin was purchased from Yucca Enterprises Mumbai . Hplc grade Acetonitrile and orthophosphoric acid was purchased from Hi Media Pvt Limited Mumbai India.Tween 80,soyalecithin and ethanol was sourced from kle college of pharmacy belagavi,India

Instrumentation

For this study, we employed the Agilent 1220 Infinity II HPLC system (model LC-20AD), a high-performance device manufactured in Japan. The system was equipped with a G7111A quaternary pump

for solvent delivery and a G7129A degasser to remove gas bubbles from the mobile phase. An integrated G7115A auto-injector facilitated automated sample introduction, while a photodiode array (PDA) detector was used for compound detection and quantification. Data acquisition and analysis were conducted using the Advanced OpenLab CDS software, which served as the primary platform for chromatographic data interpretation.

Chromatographic separation was achieved using a high-quality Phenomenex Luna C18(2) analytical column (250 mm × 4.6 mm, 5 µm particle size), manufactured by Phenomenex Inc., USA. This column provided high precision and dependable performance for chromatographic analysis.

The mobile phase consisted of acetonitrile and 0.1% orthophosphoric acid in a 90:10 (v/v) ratio, delivered at a constant flow rate of 1.0 mL/min. To prevent air bubbles from interfering with the analysis, the mobile phase was filtered through a 0.45 µm PVDF membrane filter (Millex HV, Millipore, USA) and degassed using ultrasonic treatment.

Throughout the experiment, the column temperature was maintained at 35 °C, and the injection volume was consistently set to 10 µL. Detection of Embelin was performed at a wavelength of 291 nm, ensuring accurate and reproducible results [15].

Preparation of 0.1% Ortho phosphoric Acid

In preparation of 0.1 % Ortho phosphoric acid as a mobile phase in High Performance Liquid Chromatography .A 500 ml volumetric flask was used to add 0.5 ml of OPA.The flask was then filled upto the 500ml mark with Millipore water and 2 drops of Triethyl amine was added to adjust the ph of the solution. The prepared mixture was subjected to sonication for 10 minutes to ensure thorough mixing through the application of ultrasonic sound waves. Following sonication, the solution was filtered using a 0.45 µm membrane filter to remove particulate matter. This filtration step was essential to ensure the clarity and cleanliness of the solution, making it suitable for subsequent HPLC analysis.

Preparation of standard stock solution

Stock Solution:1mg/ml solution of embelin was prepared using 10 ml volumetric flask to dissolve in acetonitrile for Hplc Analysis.

A working solution of Embelin was prepared by transferring 8ml of Acetonitrinle and 2ml of aqueous phase containing OPA to 10 ml volumetric flask.

Analytical Method Validation

The developed method was validated according to ICH guidelines for key analytical parameters, including specificity, precision, linearity, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ) and stability degradation studies.

Linearity

To assess the linearity of the method, Embelin was prepared at concentrations ranging from 2 to 10 µg/mL. Five replicate solutions were prepared for each concentration. A calibration curve was constructed by plotting the peak area against the corresponding concentration levels.

Accuracy

Recovery studies were conducted to evaluate the accuracy of the method, with average percentage recovery serving as the key criterion. Embelin standard solutions were analyzed in triplicate at three concentration levels: 50%, 100%, and 150% of the target concentration. The results were expressed as percentage recovery, calculated based on the amount of Embelin recovered relative to the amount initially added.

Precision

To evaluate the precision of the method, both intra-day (repeatability) and inter-day (intermediate precision) studies were performed. Duplicate analyses of standard solutions and sample solutions were conducted on the same day and on different days. The percentage relative standard deviation (%RSD) was calculated for both sets of results. The low %RSD values demonstrated that the method exhibited acceptable precision, in accordance with ICH guidelines.

LOD and LOQ

The sensitivity of an analytical method is characterized by its limit of detection (LOD) and limit of quantification (LOQ). According to ICH guidelines, the LOD is defined as the lowest amount of analyte in a sample that can be detected but not necessarily quantified, while the LOQ represents the lowest amount that can be quantitatively determined with acceptable precision and accuracy.

The ICH outlines three approaches for determining LOD and LOQ: (1) visual evaluation, (2) signal-to-noise ratio, and (3) calculation based on the standard deviation of the response and the slope of the calibration curve. In this study, the third approach was used.

System Suitability

System suitability testing is an essential part of HPLC method validation, ensuring the chromatographic system operates correctly and produces reliable analytical results. To verify system performance, six replicate injections of the Embelin sample were performed. Key parameters such as retention time, peak area, number of theoretical plates, and tailing factor were evaluated. These metrics confirmed the system's precision and overall suitability for the intended analysis.

Ruggedness

Ruggedness testing was performed to evaluate the method's reliability under slight, deliberate variations in experimental conditions. This assessment demonstrates the stability and robustness of the analytical technique when subjected to changes such as different analysts, laboratories, or minor procedural adjustments. Consistent results under these conditions indicate the method's dependability and suitability for routine analysis.

Robustness

Robustness was evaluated to determine the method's reliability and stability under small, intentional variations in experimental conditions. In this study, robustness was assessed by altering the detection wavelength to 288 nm and 291 nm, adjusting the flow rate to 0.9 mL/min and 1.1 mL/min, and modifying the mobile phase composition to 88:12 and 92:8 (acetonitrile:0.1% orthophosphoric acid, v/v). The method demonstrated consistent performance under these conditions, with %RSD values remaining below 2%. These results confirm the method's robustness and its ability to deliver reliable and reproducible outcomes despite minor procedural changes.

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability of the pharmaceutical samples under various stress conditions, including acid and base hydrolysis, oxidation, and photodegradation. These tests are essential for identifying potential degradation products and assessing their potential interference with the active compound. Such evaluations provide insight into the inherent stability of the active pharmaceutical ingredient and help ensure the specificity of the analytical method.

Acid-base degradation

To experiment, take 2 ml of the embelin standard solution and mix it with 2 ml of 0.1 Normal Hydrochloric Acid (HCL) in vials designated for testing acid breakdown. Repeat this by mixing another 2 ml of the embelin standard solution with 2 ml of 0.1 Normal Sodium Hydroxide (NaOH) in separate vials for testing base breakdown. Pour these mixtures into a 10 ml measuring flask. Add enough ACN to bring the total volume to 10 ml, ensuring the chemicals are thoroughly combined for the experiment. Two hours of heating occurred for the prepared solutions within 60 to 80°C. The procedure assesses how compounds maintain their integrity in basic environments that could occur in formulation or intestinal processes while creating acidic scenarios that may arise during stomach passage or manufacturing steps requiring acidic media.

Photolytic degradation

For the photodegradation study, 2 mL of standard embelin solution was transferred into a 10 mL clear volumetric flask and exposed to long-wavelength UV light for two hours. After exposure, the solution was diluted to volume with acetonitrile (ACN). An appropriate volume of the resulting solution was then injected into the HPLC system for analysis.

Thermal degradation

For the thermal degradation study, 2 mL of standard Embelin solution was placed in a 10 mL volumetric flask and diluted to volume with acetonitrile (ACN). The solution was then subjected to heating at 80 °C for two hours using a rotary bath sonicator under controlled conditions. After the treatment, the solution was analyzed to assess the extent of thermal degradation.

Oxidative degradation

Oxidative degradation was performed using a 30% hydrogen peroxide solution. In this procedure, 2 mL of standard embelin solution was added to a 10 mL volumetric flask, followed by the addition of 2 mL of 30% hydrogen peroxide. The solution was then diluted to volume with an appropriate solvent and subjected to oxidative stress by incubating it at 60–80 °C for two hours. This study aimed to evaluate the stability of the compound under oxidative conditions and to observe potential degradation pathways resulting from exposure to atmospheric oxygen or similar environmental factors.

Validation of Analytical Methods

The developed HPLC method was approved for routine laboratory use after thorough validation, addressing key performance parameters including accuracy, precision, robustness, limits of detection and quantification, linearity, and range. Validation results confirmed that the method complies with the ICH Q2(R1) guidelines, demonstrating its reliability and suitability for producing consistent and accurate analytical results in standard laboratory conditions.

Method Development

Several mobile phase compositions were evaluated to develop a reliable HPLC method for the detection of Embelin. A validated reverse-phase HPLC (RP-HPLC) method with selective detection was established, demonstrating high sensitivity and specificity for Embelin analysis. Optimal chromatographic performance was achieved using a mobile phase consisting of acetonitrile and 0.1% orthophosphoric acid in Milli-Q water in a 90:10 (v/v) ratio. This composition provided excellent peak shape, optimal resolution of Embelin, and acceptable back pressure, with a retention time of approximately 7 minutes at a flow rate of 1.0 mL/min. The detection wavelength for Embelin was set at 291 nm, corresponding to its maximum absorbance.

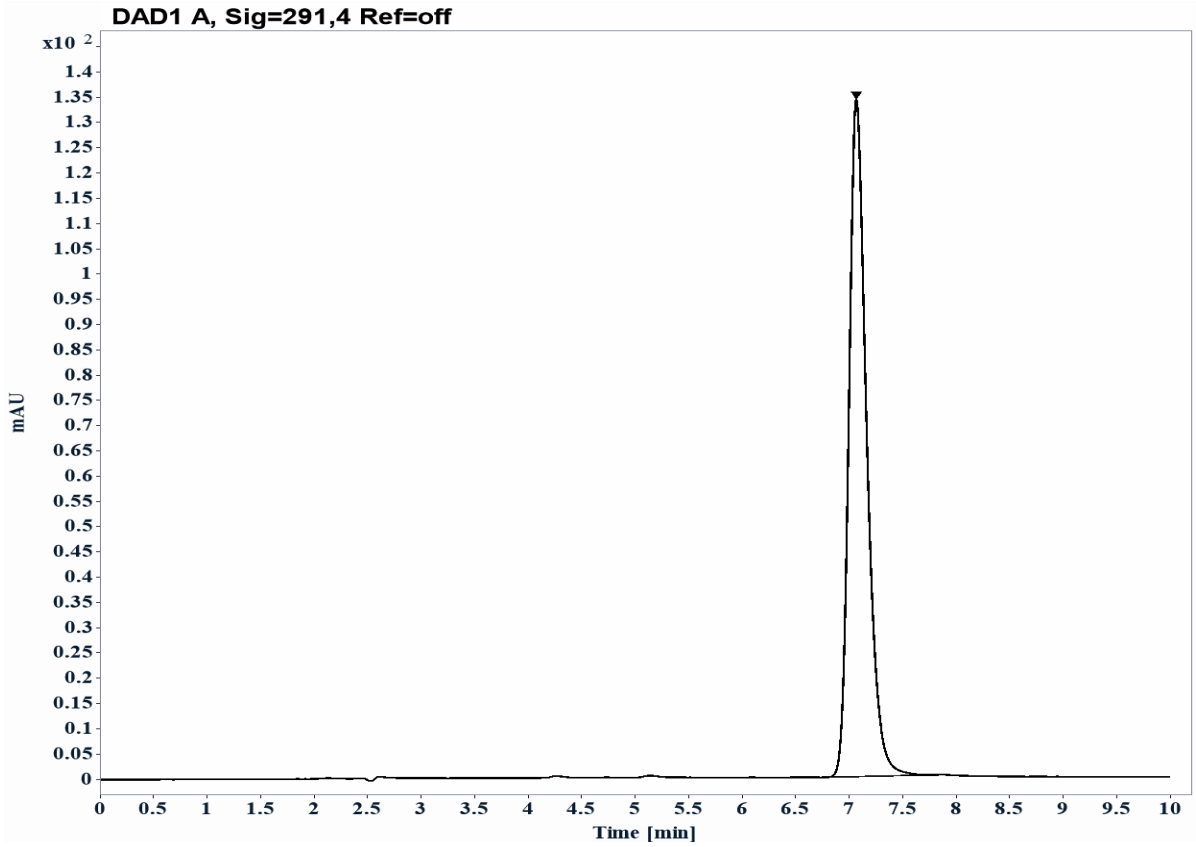


Figure 2 Embelin Standard Chromatogram

Linearity

Linearity studies for the Embelin analyte were performed using five different concentration levels, ranging from 2 to 10 µg/mL. Calibration curves were constructed by plotting peak area against concentration, as shown in *Figure X* (replace with actual figure number). The results demonstrated a strong linear correlation, with a correlation coefficient (R^2) of 0.9988, indicating excellent linearity within the tested range. This high degree of correlation confirms the method's reliability and suitability for quantitative analysis, ensuring accurate measurement of Embelin concentrations across the specified range.

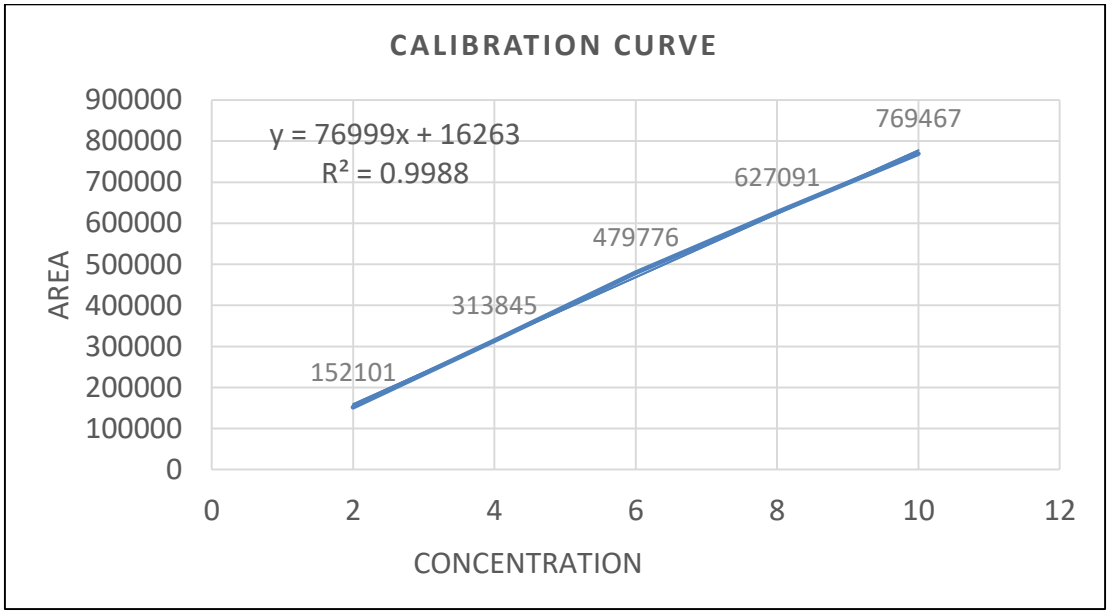


Figure 3 Calibration curve of Embelin

Accuracy

Accuracy was evaluated through recovery studies at three concentration levels: 50%, 100%, and 150% of the target analyte concentration. Triplicate samples were prepared at each level by spiking known amounts of Embelin into the sample matrix. The percentage recoveries obtained were 88.28% at 50%, 78.49% and 107.18% as detailed in Table 3. These results fall within the generally accepted recovery range for assay methods, confirming the method's accuracy in accordance with ICH Q2(R1) guidelines.

Table 1 Accuracy

Accuracy%	Theoretical (µg/mL)	Actual (µg/mL)	%Recovery
50%	6	5.95	88.28
100%	8	7.87	78.49
150%	10	10.11	107.18

Precision

The precision of the developed HPLC method was evaluated through repeatability and intermediate precision studies. Repeatability was assessed by performing six replicate injections of a 100 µg/mL Embelin solution under the same operating conditions.

Intermediate precision was evaluated by analyzing the same concentration of Embelin on different days and by different analysts. The %RSD values obtained from these studies were consistently below 2%, demonstrating the method's robustness against variations in analytical conditions. These results comply

with the ICH Q2(R1) guidelines, which recommend that %RSD values should not exceed 2% for assay methods, confirming the method's precision and suitability for routine analysis.

Table 2 Intraday and Interday

Concentration (µg/mL)	Intraday	Interday
2		
Mean ±SD	663.78±0.75	370.4±0.77
RSD%	1.81%	0.555%
6		
Mean ±SD	1087.6±0.30	1069.56±0.27
RSD%	0.027%	0.025%
8		
Mean ±SD	1353.1±0.94	1352.73±0.51
RSD%	0.055%	0.025%

LOD and LOQ

The developed HPLC method demonstrated high sensitivity for Embelin detection. The Limit of Detection (LOD) was determined to be 0.111 µg/mL, and the Limit of Quantification (LOQ) was 0.555 µg/mL. These values were calculated using the standard deviation of the response (σ) and the slope (S) of the calibration curve, applying the formulas: LOD = 3.3 × (σ/S) and LOQ = 10 × (σ/S), in accordance with ICH Q2(R1) guidelines. The low LOD and LOQ values confirm the method's suitability for detecting and quantifying Embelin at trace levels, making it appropriate for applications requiring high sensitivity.

Table 3 LOD and LOQ

Drug	LOD	LOQ
Embelin	0.111µg/mL	0.555 µg/mL

System Suitability

System suitability tests were conducted to verify critical chromatographic parameters, ensuring the HPLC system's performance meets the required analytical standards. A representative chromatogram was analyzed, and six replicate injections of the Embelin standard solution were performed. Key parameters evaluated included retention time, theoretical plate count, peak asymmetry (tailing factor), and the relative standard deviation (%RSD) of peak areas.

Table 4 System Suitability Parameters

SR.NO	System Suitability	EMBELIN	
		Mean ±SD	%RSD
1	Peak	1774.5±0.75	0.041%
2	Retention Time	7.00±0.05	1.374%
3	Theoretical Time	2975.04±0.62	0.020%
4	Tailing factor	1.338±0.004	0.155%

Robustness

The objective of this assessment was to evaluate the method's robustness its capacity to remain unaffected by small, deliberate variations in experimental conditions. To investigate this, critical parameters such as mobile phase composition, detection wavelength, and flow rate were intentionally altered. Specifically, adjustments were made to the mobile phase blend, wavelength, and flow velocity.

Despite these changes, the method exhibited strong performance, with relative standard deviation (%RSD) values remaining below 2%.

Table 5 Robustness

Conditions Changed	Changes Mode	AUC±SD	%RSD
Change in flow rate (ml/min)	0.8ml/min	1551.78±0.83	0.333%
	1.2ml/min	1551.78±1.08	0.060%
Change in wavelength (nm)	289	1551.78±0.35	0.019%
	291	1551.78±1.00	0.055%
Change in Mobile	88:12	1551.78±0.30	0.017%

phase ratio (v/v)	92:08	1551.78±1.17	0.065%

Ruggedness

Ruggedness refers to the ability of an analytical method to maintain consistent performance when subjected to small, deliberate variations in experimental conditions. It demonstrates the stability and reliability of the method across different analysts, laboratories, and slightly altered experimental parameters.

Table 6 Ruggedness parameters

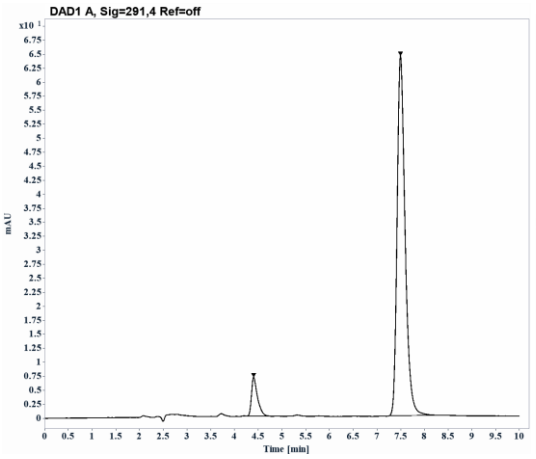
Concentration (µg/mL)	Analyst 1	Analyst 2
6	1033.4	1033.8
	1033.4	1033.1
	1033.7	1033.9
	1033.8	1033.3
	1033.6	1033.5
	1033.2	1033.9
Mean	1033.55	1033.58
Standard Deviation	0.11	0.23
%RSD	0.010%	0.021%

Forced degradation studies

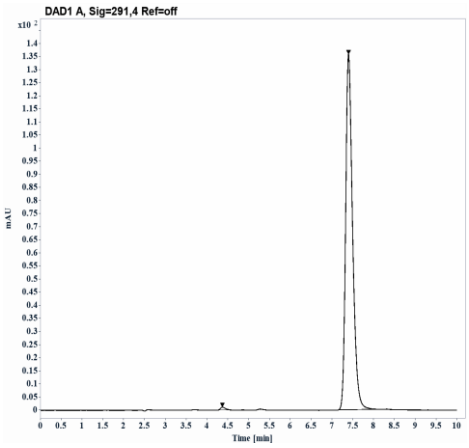
The study demonstrates that the drug's peak and those of its degradation products are well-resolved, confirming the suitability of the proposed analytical method. In the acid degradation study, Embelin exhibited a degradation rate of 37.9%**,** indicating moderate breakdown. Similarly, in the alkaline (base) degradation study, comparable degradation rates were observed along with the appearance of minor degradation peaks. Under oxidative stress, both major and minor peaks were significantly reduced, and Embelin again showed signs of degradation. In the photolytic degradation study, Embelin displayed a 42% degradation rate.

Table 7 Forced degradation studies

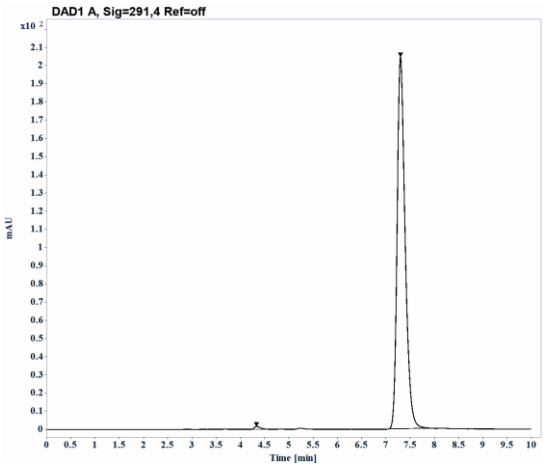
Stressed Degradation Condition	%Drug Degradation
Acid	37.9%
Base	18.2%
Hydrolysis	24.8%
Thermal	4.98%
Photolytic	42.3%



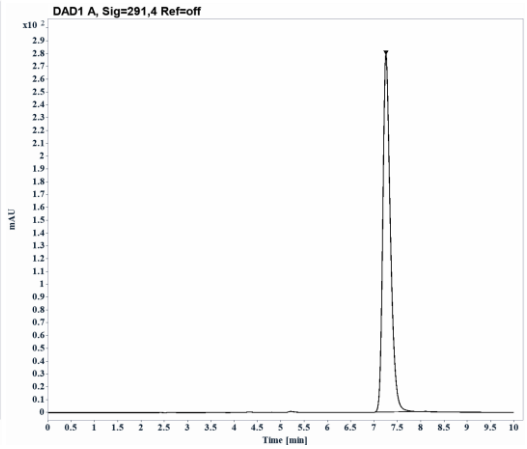
Acid



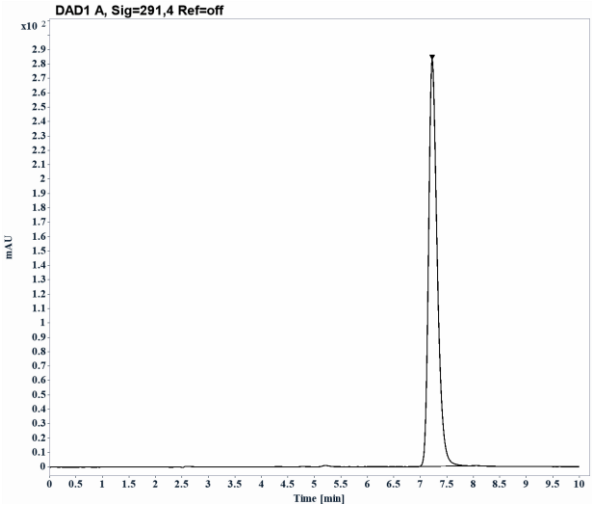
Base



Hydrolysis



Thermal



Photolytic

Formulation

Using the optimized method, embelin in the embelin loaded transethosomes developed in this study was successfully quantified through analysis of peak area and method sensitivity. The results were satisfactory, demonstrating high accuracy and low percentage coefficients of variation, indicating the method's reliability. Furthermore, this study contributes to the field by supporting the potential applicability of the developed method in routine analytical practices**.**The Hplc chromatogram for embelin loaded transethosomes is shown in figure 4.

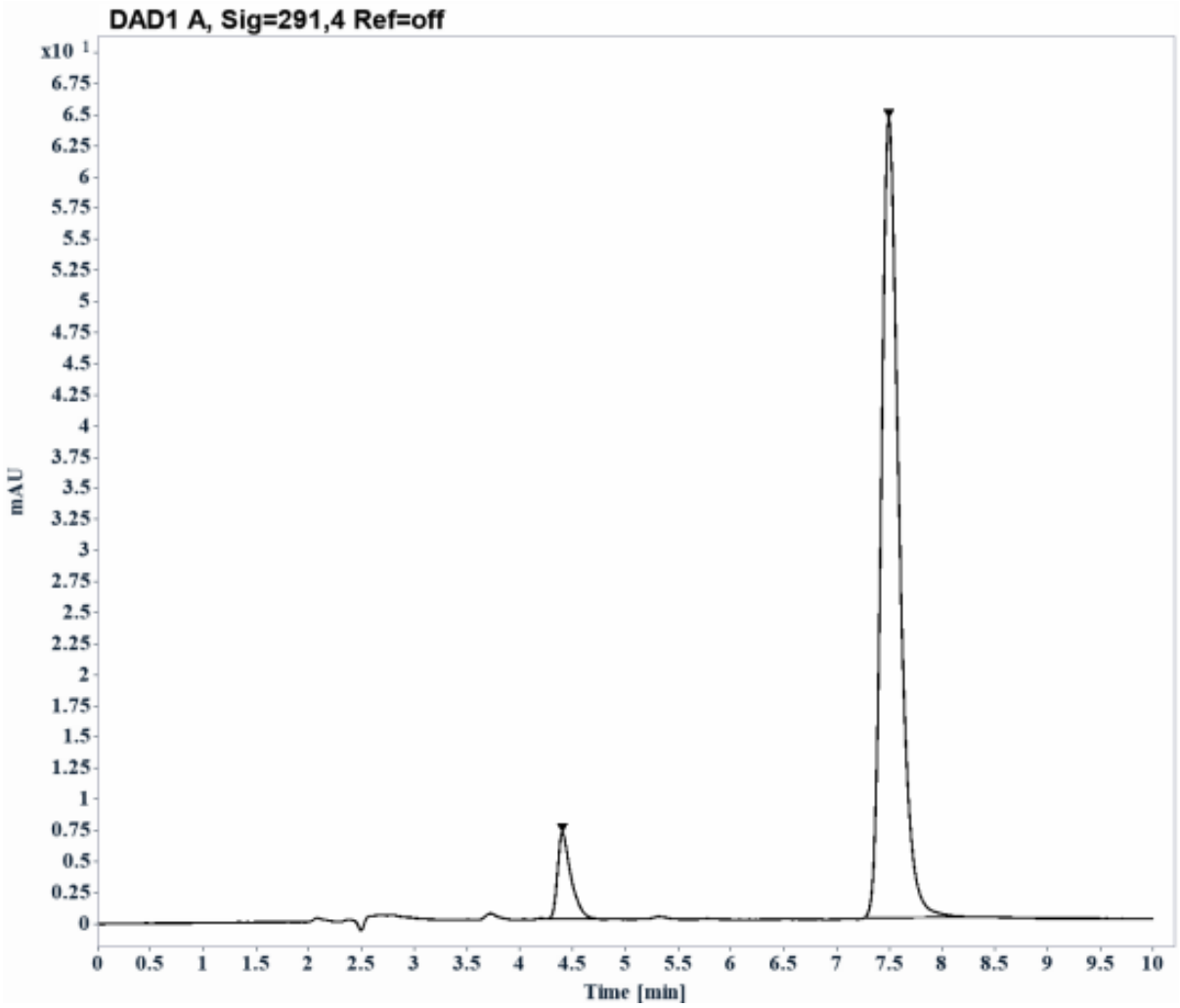


Figure 4 Hplc chromatogram of formulation

This chromatogram presents the results of an HPLC analysis conducted at 291 nm using a Diode Array Detector (DAD**)**. The X-axis represents retention time (minutes**)**, while the Y-axis shows absorbance intensity (mAU**)**. A prominent peak observed at approximately **7** minutes corresponds to the main compound in the sample. The height and sharpness of this peak suggest a high concentration and efficient detection of the target analyte. Additionally, the symmetry and narrow width of the peak indicate effective separation and good column performance.

In contrast, the smaller peaks appearing before and after the main peak may represent impurities, degradation products, or related compounds. These observations support the method's capability for detecting both the principal component and any secondary constituents in the sample.

2. Conclusion

This study successfully developed and validated a robust RP-HPLC method for the quantification embelin in both pure and pharmaceutical forms. In accordance with ICH Q2 (R1) guidelines, the method demonstrated excellent accuracy, precision, and sensitivity. Optimal chromatographic conditions included a 90% organic phase, a flow rate of 1 mL/min, and a column temperature of 30 °**C**, resulting in efficient separation with a retention time of approximately 7 minutes.

The method exhibited strong linearity (R^2 = 0.9988), high recovery rates (ranging from 98.49% to 101.18%), and appropriate LOD (0.111 µg/mL) and LOQ (0.555 µg/mL) values. Forced degradation studies revealed that Embelin is susceptible to degradation under acidic**,** basic, oxidative, and photolytic conditions, confirming the method's stability-indicating capability.

References

1.Joy B, Nishanth Kumar S, Soumya MS, Radhika AR, Vibin M, Abraham A. Embelin (2,5-dihydroxy-3-undecyl-p-benzoquinone): a bioactive molecule isolated from Embelia ribes as an effective photodynamic therapeutic candidate against tumor in vivo. Phytomedicine. 2014 Sep 25;21(11):1292-7.
2. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015 Jan;30(1):11-26.
3. Joshi R, Kamat JP, Mukherjee T. Free radical scavenging reactions and antioxidant activity of embelin: biochemical and pulse radiolytic studies. Chemico-biological interactions. 2007 Apr 25;167(2):125-34.
4.Poojari, Radhika. "Embelin–a drug of antiquity: shifting the paradigm towards modern medicine." Expert opinion on investigational drugs 23.3 (2014): 427-444.
5.Miot LD, Miot HA, Silva MG, Marques ME. Physiopathology of melasma. An Bras Dermatol. 2009;84:623–635. doi: 10.1590/s0365-05962009000600008
6.Sarkar R, Ghunawat S, Narang I, Verma S, Garg VK, Dua R. Role of broad-spectrum sunscreen alone in the improvement of melasma area severity index (MASI) and Melasma Quality of Life Index in melasma. J Cosmet Dermatol. 2019 Aug;18(4):1066-1073.
7.Yeung H, Kahn B, Ly BC, Tangpricha V. Dermatologic Conditions in Transgender Populations. Endocrinol Metab Clin North Am Ching D, Amini E, Harvey NT, Wood BA, Mesbah Ardakani N. Cutaneous tumoural melanosis: a presentation of complete regression of cutaneous melanoma. Pathology. 2019.
8.Roberts WE, Henry M, Burgess C, Saedi N, Chilukuri S, Campbell-Chambers DA. Laser Treatment of Skin of Color for Medical and Aesthetic Uses With a New 650-Microsecond Nd:YAG 1064nm Laser. J Drugs Dermatol. 2019 Apr 01;18(4):s135-137.
9.Rendon MI. Hyperpigmentation Disorders in Hispanic Population in the United States. J Drugs Dermatol. 2019 Mar 01;18(3):s112-114.
10.Zubair R, Lyons AB, Vellaichamy G, Peacock A, Hamzavi I. What's New in Pigmentary Disorders. Dermatol Clin. 2019 Apr;37(2):175-181.

11.Passeron T, Genedy R, Salah L, Fusade T, Kositratna G, Laubach HJ, Marini L, Badawi A. Laser treatment of hyperpigmented lesions: position statement of the European Society of Laser in Dermatology. J Eur Acad Dermatol Venereol. 2019 Jun;33(6):987-1005.
12.Kwon SH, Na JI, Choi JY, Park KC. Melasma: Updates and perspectives. Exp Dermatol. 2019 Jun;28(6):704-708
13.Juhasz MLW, Levin MK. The role of systemic treatments for skin lightening. J Cosmet Dermatol. 2018 Dec;17(6):1144-1157.
14.Madhavan, Soumya N., Ranjith Arimboor, and C. Arumughan. "RP-HPLC-DAD method for the estimation of embelin as marker in Embelia ribes and its polyherbal formulations." Biomedical chromatography 25, no. 5 (2011): 600-605.
15.Vijayan, K.P.R., Raghu, A.V. Embelin: an HPTLC method for quantitative estimation in five species of genus Embelia Burm. f.. Futur J Pharm Sci **7**, 55 (2021).