Development and Validation of a UV-Spectrophotometric Method for Analysis of Madecassoside in Centella asiatica: A Simple and Reliable Analytical Approach

Ronak Dsouza[1], Ganesh Patil[2], Vittal Gavali[3], Veena Vithal Patil[4], Tanvi Bamburge[5], Shailendra Sanjay Suryawanshi[6], Dr. Shailendra Sanjay Suryawanshi[7]

[1,2,3,4,5,6]*Department of Pharmaceutical Analysis, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.*

[7]*Department of Pharmaceutical Analysis, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India*

***Abstract*—The present study aimed to develop and preliminarily validate a simple UV-spectrophotometric method for the analysis of Madecassoside in Centella asiatica. The method was developed using methanol as the solvent, with maximum absorption observed at 210 nm. A Shimadzu UV-1800 spectrophotometer was employed for spectral measurements. The calibration curve was established over a concentration range of 100–500 µg/mL, demonstrating good linearity with a regression equation of y = 0.002008x − 0.0304 and a coefficient of determination (R^2) of 0.99814. Accuracy studies showed percentage recoveries ranging from 98.00% to 99.17%, with a mean recovery of 98.64%. Preliminary precision results indicated %RSD values below 2%. The estimated limit of detection and limit of quantification were 26.01 µg/mL and 78.83 µg/mL, respectively. The proposed method offers a straightforward and economical approach for preliminary Madecassoside analysis, with further studies required to establish matrix specificity in herbal extracts.**

***Index Terms*—Madecassoside, Centella asiatica, UV-Visible spectrophotometry, Analytical method validation, Herbal standardization.**

I. INTRODUCTION

Medicinal plants are important sources of bioactive phytochemicals and have been widely investigated for their potential therapeutic applications. Their increasing incorporation into pharmaceutical, nutraceutical and cosmeceutical products has highlighted the importance of reliable analytical procedures for ensuring identity, purity, potency and consistency. Standardization of herbal materials is particularly important because their phytochemical composition can vary with geographical origin, cultivation conditions, harvesting period, processing and extraction procedures [1,2]. Centella asiatica (L.) Urb., belonging to the family Apiaceae, is a medicinal herb distributed across tropical and subtropical regions. It has been traditionally used in different healthcare systems and has attracted scientific attention because of its reported wound-healing, anti-inflammatory, antioxidant and neuroprotective properties [1,2]. Its phytochemical composition includes pentacyclic triterpenoids, triterpenoid glycosides, flavonoids and other phenolic constituents. Madecassoside is an important triterpenoid glycoside and characteristic phytochemical marker of Centella asiatica. It is structurally related to other centellosides, particularly Asiaticoside, and their corresponding aglycones, Madecassic acid and Asiatic acid. These phytochemicals have been investigated for their potential pharmacological activities, including their roles in skin repair, inflammation modulation and tissue regeneration [2,3].

Quantitative estimation of Madecassoside is relevant to the quality evaluation of raw herbal materials, standardized extracts and herbal formulations. Analytical methods reported in the literature include high-performance liquid chromatography (HPLC) and HPLC coupled with UV detection. Schaneberg et al. described an HPLC method for quantitative determination of six triterpenes in Centella asiatica, including Madecassoside [4]. Rafamantanana et al.

also reported an HPLC-UV method for simultaneous quantification of four major triterpenic constituents in the plant [5].

Although chromatographic methods provide separation of individual phytochemical constituents, they require specialized instrumentation, chromatographic columns, mobile phases and additional analytical resources. UV-visible spectrophotometry offers a comparatively simple and accessible analytical technique for quantitative estimation when the analyte exhibits suitable UV absorption and adequate selectivity can be established. The development of a direct UV-spectrophotometric method for Madecassoside requires careful consideration of its solvent compatibility, absorption characteristics and potential interference from other UV-absorbing constituents. Measurements in the low UV region particularly require appropriate blank correction and evaluation of solvent and matrix absorbance. The International Council for Harmonisation (ICH) Q2(R2) guideline provides recommendations for evaluating analytical procedure performance, including specificity, accuracy, precision, linearity, range and detection and quantification limits [6]. The complementary ICH Q14 guideline describes scientific principles for analytical procedure development [7].

Considering these requirements, the present investigation was undertaken to develop a UV-spectrophotometric procedure for the analysis of Madecassoside using methanol as the analytical solvent. The absorption maximum was identified at 210 nm, and the calibration range was established between 100 and 500 µg/mL. The analytical procedure was investigated through calibration regression, recovery and precision studies, with the objective of exploring its potential application in the quality assessment of Centella asiatica extracts.

II. MATERIALS AND METHODS

Chemicals and reagents

Madecassoside reference standard was procured from Sunpure Extracts Private Limited, a Government of India-recognized Star Export House, Sunpure House, Plot No. 11/29, Sahibabad Industrial Area, Sahibabad, Ghaziabad, Uttar Pradesh – 201010, India. The procured Madecassoside was used as the reference marker for analytical method development, calibration and validation studies. Methanol was used as the solvent for preparation of standard and working solutions. The Centella asiatica extract was used for preliminary application of the developed procedure.

Instrumentation

Spectrophotometric analysis was performed using a Shimadzu UV-1800 UV-visible spectrophotometer equipped with UV-Probe software. The quartz cuvettes with a 1 cm optical path length were used for absorbance measurements.

Solubility assessment

The solubility of Madecassoside was initially investigated in selected solvents. Methanol was identified as a suitable solvent based on the observed dissolution of the reference standard and was selected for further analytical investigations.

Preparation of standard solution

An accurately weighed quantity of Madecassoside reference standard was transferred into a calibrated volumetric flask and dissolved in methanol. The volume was adjusted using the same solvent to obtain a standard stock solution of known concentration. Suitable dilutions of the stock solution were prepared for spectral analysis and calibration studies.

Determination of wavelength of maximum absorption

An appropriate working solution of Madecassoside was prepared in methanol and scanned in the UV region against methanol as the blank. The absorption spectrum was recorded, and the wavelength corresponding to maximum absorbance was identified. The maximum absorption wavelength was found to be 210 nm and was selected for subsequent quantitative analysis.

Preparation of calibration standards

Working standard solutions were prepared by suitable dilution of the stock solution to obtain concentrations of 100, 200, 300, 400 and 500 µg/mL. The absorbance of each solution was measured at 210 nm using methanol as the blank. A calibration curve was constructed by plotting concentration against the corresponding absorbance. Linear regression analysis was performed to determine the slope, intercept, correlation coefficient and coefficient of determination.

Preparation of herbal extract sample

An accurately weighed quantity of 10 mg of Centella asiatica extract was transferred into a 10 mL volumetric flask. Methanol was added as the extraction solvent, and the mixture was subjected to sonication for 10 minutes to facilitate dissolution and extraction of the phytochemical constituents. The resulting solution was filtered to remove insoluble material. Suitable serial dilutions were subsequently prepared to obtain a sample solution intended for spectrophotometric analysis. The spectrum was obtained using methanol as the blank.

Method validation

The analytical procedure was investigated using selected validation characteristics based on ICH Q2(R2) [6] (8-53)

Linearity

Linearity was investigated over the concentration range of 100–500 µg/mL using five calibration levels. Absorbance values were plotted against corresponding concentrations, and linear regression analysis was performed.

The regression equation was expressed as: $y = mx + c$. Where y represents absorbance, x represents concentration, m represents the slope and c represents the intercept.

Accuracy

Accuracy was evaluated using the standard addition approach at 80%, 100% and 120% levels. A known quantity of Madecassoside was added to a sample containing an original concentration of 10 µg/mL. The percentage recovery was calculated using:

% Recovery = [(Total amount found − Original amount present) / Amount added] × 100

Precision

Precision was investigated through intraday and interday measurements at three concentration levels. For intraday precision, measurements were performed within the same day under similar experimental conditions. Interday precision was investigated on different days. The results were expressed as mean absorbance, standard deviation and percentage relative standard deviation. %RSD = (Standard deviation / Mean) × 100

Limit of detection and quantification

The limits of detection and quantification should be established using the standard deviation of the analytical response and the slope of the calibration curve, according to ICH recommendations. LOD = $3.3\sigma/S$, LOQ = $10\sigma/S$. Where σ represents the standard deviation of the response and S represents the calibration slope.

Statistical analysis

Experimental results were expressed as mean, standard deviation and percentage relative standard deviation wherever replicate observations were available. Calibration regression and recovery statistics were calculated using the experimental observations.

III. RESULTS AND DISCUSSION

Solubility assessment

The preliminary solubility investigation demonstrated that Madecassoside was soluble in methanol under the experimental conditions employed. Methanol was therefore selected for preparing standard and working solutions. The selection of a suitable solvent is important in spectrophotometric method development because the solvent should provide adequate analyte solubility without causing significant interference at the selected analytical wavelength.

Determination of wavelength of maximum absorption

The UV absorption spectrum of Madecassoside (Figure 1) was recorded using methanol as the blank. The maximum absorption wavelength was identified at 210 nm. This wavelength was selected for subsequent quantitative measurements [4,5].

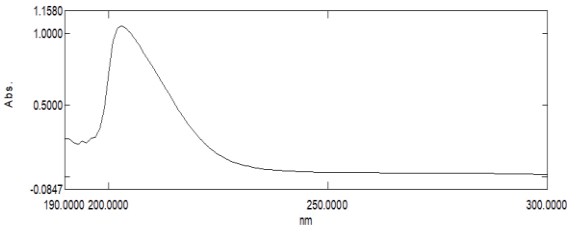


Figure 1. UV absorption spectrum of Madecassoside showing the absorption maximum at 210 nm.

Linearity study

The linearity of the proposed procedure was investigated using five concentration levels ranging

from 100 to 500 µg/mL. The absorbance values increased progressively with increasing analyte concentration. The calibration data demonstrated a strong positive linear relationship between Madecassoside concentration and absorbance (Table 1, Table 2).

Table 1. Calibration data of Madecassoside at 210 nm

Concentration (µg/mL)	Absorbance
100	0.184
200	0.366
300	0.557
400	0.764
500	0.989

The regression equation obtained was: $y = 0.002008x - 0.0304$. The correlation coefficient was 0.99907, and the coefficient of determination was 0.99814.

Table 2. Regression analysis of Madecassoside

Parameter	Result
Analytical wavelength	210 nm
Linearity range	100–500 µg/mL
Slope	0.002008
Intercept	−0.0304
Regression equation	$y = 0.002008x - 0.0304$
Correlation coefficient (r)	0.99907
Coefficient of determination (R^2)	0.99814

The obtained regression results demonstrate a strong linear association between concentration and absorbance. However, assessment of residual distribution and replicate calibration measurements would provide additional evidence regarding the suitability of the regression model.

Accuracy study

Accuracy was evaluated using the standard addition approach at 80%, 100% and 120% levels. The original sample concentration was 100 µg/mL, and known amounts of Madecassoside standard were added. The percentage recoveries were 98.75%, 98.00% and 99.17%, respectively (Table 3, Table 4)

Table 3. Accuracy and percentage recovery of Madecassoside

Level	Standard added (µg/mL)	Recovery (%)
80%	80	98.75
100%	100	98.00
120%	120	99.17

Table 4. Statistical summary of recovery results

Parameter	Result
Number of recovery levels	3
Mean recovery (%)	98.64
SD	0.59
%RSD	0.60

The recovery results indicate that the measured amounts were close to the corresponding added amounts. The overall mean recovery of 98.64% suggests promising analytical accuracy under the investigated conditions. The final validation report should also include replicate recovery measurements at each concentration level to demonstrate within-level variability.

Precision study

Precision was assessed through intraday and interday measurements at three concentration levels. The results were evaluated using mean absorbance, SD and recalculated %RSD (Table 5, Table 6).

Table 5. Intraday precision study

Concentration (µg/mL)	Mean absorbance	SD	Calculated %RSD
100	0.190	0.001	0.53
300	0.580	0.001	0.17
500	1.000	0.003	0.30

Table 6. Interday precision study

Concentration (µg/mL)	Mean absorbance	SD	Calculated %RSD
100	0.203	0.001	0.49
300	0.590	0.001	0.17
500	1.050	0.016	1.52

The calculated %RSD values were low, indicating limited variability in the reported precision measurements. The intraday values ranged from 0.17% to 0.53%, whereas interday values ranged from 0.17% to 1.52%. These findings indicate promising repeatability and intermediate precision. However, the final assessment should be based on the original replicate readings and confirmation of the precision concentration units.

LOD and LOQ

The sensitivity of the proposed UV-spectrophotometric method was preliminarily estimated using the standard deviation of the regression response and the slope of the calibration

curve. The calculated LOD and LOQ were 26.01 µg/mL and 78.83 µg/mL, respectively. These results provide an initial estimate of the analytical sensitivity of the proposed method. The calculated LOQ is below the established calibration range of 100–500 µg/mL (Table 7).

Table 7: Determination of LOD and LOQ of the proposed UV-spectrophotometric method

Parameter	Result
Regression slope	0.002008
Residual standard deviation	0.015828
Limit of Detection (LOD)	26.01 µg/mL
Limit of Quantification (LOQ)	78.83 µg/mL

Application to Centella asiatica Extract

The applicability of the developed UV-spectrophotometric method was qualitatively investigated using a Centella asiatica extract. An accurately weighed quantity of 10 mg of the extract was transferred into a 10 mL volumetric flask, followed by the addition of methanol. The mixture was sonicated for 10 minutes to facilitate dissolution and extraction of the phytochemical constituents. The resulting solution was filtered, and the filtrate was subjected to appropriate dilution before spectrophotometric analysis at 210 nm. The prepared extract exhibited UV absorption at the selected analytical wavelength, indicating the presence of UV-absorbing constituents consistent with the expected phytochemical profile. The observed spectral response demonstrated the preliminary applicability of the proposed procedure for the qualitative assessment of Centella asiatica extract. However, confirmation and quantification of Madecassoside specifically would require comparison with the reference standard and appropriate selectivity assessment.

Specificity

The specificity of the proposed UV-spectrophotometric method was evaluated by recording the absorption spectrum of the solvent blank (methanol) under the same experimental conditions used for Madecassoside analysis. The blank spectrum showed no significant absorbance or interfering signal at the selected wavelength of maximum absorption (λmax) of Madecassoside (210 nm). This indicated that methanol did not produce appreciable interference with the analytical response at the selected wavelength. Therefore, the solvent was considered suitable for use as the blank during spectrophotometric measurements.

Validation summary

Table 7. Summary of analytical method development and validation

Parameter	Experimental condition	Finding/status
Solvent	Methanol	Selected
Wavelength maximum	210 nm	Established
Linearity	100–500 µg/mL	$R^2 = 0.99814$
Accuracy	80%, 100%, 120%	Mean recovery = 98.64%
Intraday precision	Three reported levels	%RSD = 0.17–0.53
Interday precision	Three reported levels	%RSD = 0.17–1.52
LOD	ICH calculation	26.01 µg/mL
LOQ	ICH calculation	78.83 µg/mL
Specificity	Blank and Marker Analysis	Method found specific.

IV. DISCUSSION

The present investigation describes the development of a UV-spectrophotometric approach for quantification of Madecassoside, an important triterpenoid glycoside of Centella asiatica. The preliminary analytical findings established methanol as a suitable solvent and 210 nm as the maximum absorption wavelength. The calibration study demonstrated a strong linear relationship over the selected concentration range of 100–500 µg/mL. The regression equation and coefficient of determination indicated a promising concentration-response relationship. These results provide a basis for further analytical evaluation of the proposed method. The accuracy investigation demonstrated recoveries between 98.00% and 99.17%, with an overall mean recovery of 98.64%. These findings indicate that the measured concentrations were close to the expected concentrations in the standard addition experiment. The precision study also showed low reported variability, with calculated %RSD values below 2% across the investigated levels. Nevertheless, the concentration units used for precision experiments should be verified, and the original replicate readings should be retained for statistical confirmation.

A significant analytical consideration is the complex phytochemical composition of Centella asiatica. The plant contains multiple triterpenoid glycosides, flavonoids and other constituents that may exhibit overlapping UV absorption. Therefore, the absorbance measured at 210 nm cannot automatically be attributed exclusively to Madecassoside in a crude herbal extract. Earlier investigations using HPLC-UV have demonstrated the importance of chromatographic separation for quantifying individual triterpenic constituents in Centella asiatica [4,5]. Consequently, chromatographic confirmation, matrix-spiked recovery studies and comparison with a suitable reference procedure would strengthen the selectivity claims of the proposed direct UV method. Overall, the preliminary findings support further investigation of this straightforward spectrophotometric procedure. Its final suitability for routine herbal quality control will depend on completion of the remaining validation characteristics and demonstration of adequate selectivity in the herbal matrix.

V. CONCLUSION

The present study successfully established the analytical conditions for the development of a UV-spectrophotometric method for Madecassoside analysis. Methanol was selected as the suitable solvent, with maximum absorption at 210 nm. The method demonstrated linearity over the concentration range of 100–500 µg/mL, with an R^2 of 0.99814. Accuracy studies showed a mean recovery of 98.64%, while precision studies indicated low variability, with %RSD values below 2%. The preliminary LOD and LOQ were estimated as 26.01 µg/mL and 78.83 µg/mL, respectively. The observed UV absorption of the methanolic Centella asiatica extract further demonstrated the potential applicability of the proposed procedure for herbal analysis. Overall, the method offers a simple and economical approach for Madecassoside analysis, although further confirmation of specificity, robustness and matrix interference is necessary before its application in routine herbal standardization.

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