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Research Article

Validated UV-Visible Spectrophotometric Method for Quantitative Analysis of Glycyrrhizin

Bhagyashri More, Pravin Wakte, Sachin Bhusari *

University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, India

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For Correspondence:

Sachin Bhusari, University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, India

Abstract

Aim: The study aimed to develop and validate a simple, accurate, precise and cost-effective UV-Visible spectrophotometric method or the estimation of Glycyrrhizin in formulation.

Methods: A UV-Visible spectrophotometric method for Glycyrrhizin was developed using HPLC grade water. The solution was scanned across the UV-Visible range of 200-800 nm to identify its wavelength of maximum absorbance. The linearity of the method was established using six calibration standards over a concentration range of 5–75 µg/mL. Three different quality control standard solutions of the Glycyrrhizin were used for establishing the accuracy and precision of the proposed method. Proposed method was validated using ICH Q2 (R1) guidelines on the basis of accuracy, precision, robustness, limit of Detection (LOD), and limit of Quantitation (LOQ).

Results: Glycyrrhizin when dissolved in methanol and diluted by using HPLC grade water showed maximum absorbance at wavelength of 254 nm. The developed UV–Visible spectrophotometric method demonstrated excellent linearity across the concentration range of 5–75 µg/mL, with a Correlation coefficient (r^2) of 0.999. The intra-day accuracy of the proposed UV–Visible Spectrophotometric method in terms of % Difference was in the range of +0.5487 to +1.8644 whereas the inter-day accuracy was in the range of +0.6250 to + 1.9096. The intra-day precision of the proposed UV–Visible spectrophotometric method in terms of % RSD was found to be in between 0.2681 to 1.3558 whereas the inter-day precision values were in between 0.2581 to 1.3052. The variation (% RSD) during the robustness study of the proposed UV–Visible spectrophotometric method was found be below 2%. The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed UV–Visible spectrophotometric method was found to be 0.6023 µg/mL and 1.82 µg/mL respectively, ensuring adequate sensitivity for routine analysis. Proposed method was successfully used for the estimation of Glycyrrhizin in the formulation.

Conclusion: The proposed UV-Visible spectrophotometric method for estimation of Glycyrrhizin is simple, sensitive, accurate, and cost effective. It is suitable for routine analysis of Glycyrrhizin in commercial formulation.

Keywords: Glycyrrhizin, UV-Visible method, ICH Q2 (R1)

1. INTRODUCTION

The liquorice plant (*Glycyrrhiza glabra*) is a widely used medicinal herb known for its therapeutic properties. Its principal bioactive constituent is glycyrrhizin, also referred to as glycyrrhizic acid, which belongs to the class of triterpenoid saponin glycosides [1]. Structurally, glycyrrhizin comprises glycyrrhetinic acid conjugated with two glucuronic acid residues. [2]. Glycyrrhizin has a molecular formula of $C_{42}H_{62}O_{16}$ and a molecular weight of 822.93 g/mol [2].Glycyrrhizin has been reported to possess diverse pharmacological activities, including anti-inflammatory, antiviral, hepatoprotective, immunomodulatory, and antioxidant effects [3-5]. These activities contribute to its broad therapeutic potential in both traditional and modern medicine [6].

Physically, it appears as a white to pale amorphous powder and exhibits poor solubility in water while showing better solubility in organic solvents such as methanol and alcohol, which poses challenges in formulation development and quantitative estimation [7].

Due to its limited aqueous solubility and low intestinal permeability, glycyrrhizin is categorized as a Class IV compound under the Biopharmaceutics Classification System (BCS), indicating poor oral bioavailability [3]. These physicochemical limitations make it necessary to develop accurate, sensitive, and reproducible analytical methods for the estimation of glycyrrhizin in bulk drug and complex herbal formulations [8]. Various analytical techniques, including UV–Visible spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin-Layer Chromatography (HPTLC), have been reported for the determination of glycyrrhizin [9]. However, many chromatographic methods involve sophisticated instrumentation and complex sample preparation, limiting their routine use. Therefore, a simple, precise, and cost-effective UV–Visible spectrophotometric method is highly desirable

for routine quality control of glycyrrhizin in herbal and pharmaceutical products [7].

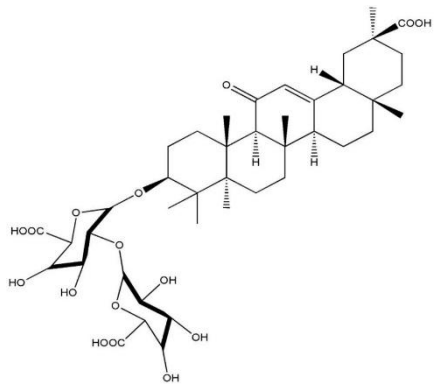

Figure 1: Molecular Structure of Glycyrrhizin

2. MATERIAL AND METHOD

2.1 Instrumentation:

The analysis was carried out using a pre-calibrated double beam UV-Visible spectrophotometer (Model UV-530, Jasco, Japan) operated using Spectra Manager software for Smooth data collection and processing. Quartz cuvettes measuring 3 cm in length and 1 cm in path length were used to measure the absorbance of the sample. All weighing procedures were conducted using an analytical balance (Essae, Vibra HT) equipped with an internal calibration system to maintain consistency and accuracy throughout the study. For complete solubilization, an ultrasonic bath (PCI Analytics, India; 6.5 L capacity) was used.

2.2 Material:

High purity Glycyrrhizin was purchased from TCI Chemicals (India) Pvt. Ltd., Chennai. The HPLC-grade methanol were purchased from Merck. All other chemicals and reagents used for analysis were of HPLC grade and sourced from trusted suppliers.

2.3 Preparation of standard stock solutions:

To prepare a stock solution of 1000 µg/mL, 10 mg of Glycyrrhizin was accurately weighed and dissolved in 10 mL of methanol in a volumetric flask, which was designated as the mother stock solution. From mother stock, an intermediate solution of 100 µg/mL was prepared by appropriate dilution with 100% HPLC-grade water and designated as Stock-I. Stock-I was further diluted using HPLC-grade water to prepare the working stock solution of 10 µg/mL (Stock-II). The Stock-II solution was used for UV–Visible spectrophotometric analysis to determine the wavelength of maximum absorption (λ_{max}) and to record the spectral characteristics of Glycyrrhizin.

2.4 Determination of wavelength of maximum absorbance (λ_{max}):

The λ_{max} of Glycyrrhizin was determined using a UV-Visible spectrophotometer. The instrument was first calibrated by setting it to auto-zero with HPLC grade water as the blank. A working standard / Stock-II solution was used for the determination of wavelength of maximum absorbance (λ_{max}). The spectrophotometer was set in Spectrum Measurement mode and the Stock-II solution of Glycyrrhizin was scanned over the wavelength range of 200 to 800 nm. The scanning process was repeated three times using a fresh Stock-II solutions.

2.5 Preparation of calibration curve:

Mother Stock solution was diluted suitably with HPLC grade water so as to achieve the solution of strength 100 µg/mL (Stock-I). Stock-I solution was diluted suitably using a HPLC grade water so as to achieve the six calibration standards viz, CAL-STD-1 (5 µg/mL), CAL-STD-2 (15 µg/mL), CAL-STD-3 (30 µg/mL), CAL-STD-4 (45 µg/mL), CAL-STD-5 (60 µg/mL) & CAL-STD-6 (75µg/mL) to cover the required range. The Spectrophotometer was set in Fixed Wavelength Measurement mode and absorbance of each CAL-STD was measured at pre-measured wavelength of maximum absorbance of 254 nm. The above mentioned procedure was repeated three times to ensure the reproducibility of the results. The results were expressed in terms of mean ± SD.

2.6 Method Validation

The UV-Visible spectrophotometric method for Glycyrrhizin was validated using ICH Q2 (R1) guidelines [10]. Developed method was assessed for its linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

2.6.1 Linearity and Range:

The linearity of the developed UV–Visible spectrophotometric method for Glycyrrhizin was evaluated using six pre-defined calibration standards, as described earlier. Calibration curves were constructed by plotting absorbance against concentration, and the data were analyzed using the linear least-squares regression method. The linearity of the method was confirmed based on the correlation coefficient (r^2) value. The range of the developed UV method was stated to be in between the upper and lower concentration limits with satisfactory linearity.

2.6.2 Accuracy:

The accuracy of the developed UV–Visible spectrophotometric method for Glycyrrhizin was evaluated using three quality control standards viz QC-STD 1, QC-STD 2 and QC-STD 3 having nominal concentrations of 7 µg/mL, 35 µg/mL, and 70 µg/mL respectively, each analyzed in triplicate and used for proposed study. Said QC-STD were analyzed for its Glycyrrhizin content using proposed UV Visible spectrophotometric method at three different time intervals in a day. Said process of QC-STDs preparation and its analysis for Glycyrrhizin content was repeated for the three consecutive days. The intra-day and inter-day accuracy of the proposed method was established in terms of % Difference which was calculated using following formula,

$$\% \text{ difference} = \frac{\text{Mean measured concentration} - \text{Nominal concentration}}{\text{Nominal concentration}} \times 100 \quad \text{.....(01)}$$

2.6.3 Precision:

The precision of the proposed UV–Visible spectrophotometric method for Glycyrrhizin was evaluated to determine the consistency and reliability of the method in terms of % Relative Standard Deviation (RSD) using the same QC STDs as of accuracy studies at concentrations of **7** µg/mL, 35 µg/mL, and 70 µg/mL. Intra-day precision was determined by analyzing the QC-STDs at three different time intervals within a single day, while inter-day precision was established by repeating the same analysis over three consecutive days. The precision of the method was confirmed as all calculated %RSD values remained within the acceptable limit of ≤ 2%. The % RSD was calculated using following formula,

$$\% \text{ RSD} = \frac{\text{Standard Deviation(SD)}}{\text{Mean}} \times 100 \ldots\ldots\ldots (02)$$

2.6.4 Robustness:

Robustness of the UV-Visible spectrophotometric method for Glycyrrhizin was assessed by introducing a small, deliberate change in the detection wavelength. Since the λ_{max} was determined to be 254 nm, the method was re-evaluated at 252 nm and 256 nm (λ_{max} ± 2 nm) to check whether such variation affected the results. Three quality control concentrations were analyzed at each adjusted wavelength, and the absorbance values were used to calculate the mean, standard deviation (SD), and percent relative standard deviation (%RSD). The robustness of the method was confirmed by deliberate minor variations in analytical conditions, with all calculated %RSD values remaining within the acceptable limit of ≤ 2%.

2.6.5 Limit of Detection (LOD):

The LOD of the developed UV method was calculated by using the following formula,

$$\text{LOD} = \frac{3.3 \times \text{SD}}{\text{S}} \ldots\ldots\ldots (03)$$

Where, SD= Standard deviation of Y-intercepts

S= Slope of the calibration curve

2.6.6 Limit of Quantitation (LOQ):

The LOQ of the developed UV method was calculated by using the following formula,

$$\text{LOQ} = \frac{10 \times \text{SD}}{\text{S}} \ldots\ldots\ldots (04)$$

Where, SD= Standard deviation of Y-intercepts

S= Slope of the calibration curve

2.7 Estimation of Glycyrrhizin in marketed formulations:

The proposed UV-Visible spectrophotometric method was applied to the determination of Glycyrrhizin in marketed Glycyrrhiza glabra tablets so as to ensure the method's suitability for routine quality control analysis of three different brands of Glycyrrhiza glabra tablets. A sample equivalent to 10 mg of Glycyrrhizin was weighed accurately, dissolved in methanol, the solutions were subjected to ultrasonication for 15 minutes using ultrasonic water bath. After sonication, solutions were filtered through 0.22 µm syringe filter and diluted to a final concentration of 50 µg/mL for analysis and dilutions were mixed thoroughly using vortex mixer. The solutions were analyzed for Glycyrrhizin content using a pre-validated UV–Visible spectrophotometric method.

3. RESULTS AND DISCUSSION

3.1 Determination of the wavelength of maximum absorbance

For accurate quantitative estimation of glycyrrhizin by UV–Visible spectrophotometry, identifying the wavelength corresponding to the maximum absorbance is required, as it ensures enhanced sensitivity and reliability of the results. In the proposed study, a 10 µg/mL glycyrrhizin solution was prepared using HPLC-grade water and scanned from 200 to 800 nm in the spectrum measurement mode. The obtained spectrum was analyzed using the instrument's software, and the wavelength showing the maximum absorbance was found to be 254 nm, which was selected for all further analytical measurements.

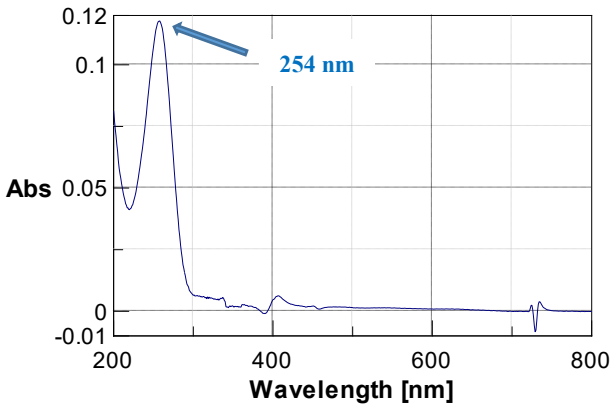

Figure 2: UV-Visible Spectra of Glycyrrhizin

3.2 Preparation of a calibration curve

For accurate quantification of glycyrrhizin using a UV-Visible spectrophotometer, it is important to establish a clear relationship between concentration and absorbance. Instead of graphical method, an equation-based calibration method provides greater precision and universal acceptance for determining unknown sample concentrations. In the present study, a calibration curve for glycyrrhizin was prepared by analyzing a series of standard solutions viz, 5 µg/mL, 15 µg/mL, 30 µg/mL, 45 µg/mL, 60 µg/mL, and 75 µg/mL. Each solution was measured three times for its absorbance at 254 nm in the fixed wavelength mode. The average absorbance values along with their standard deviations were calculated, and a calibration curve was plotted. The calibration curve served as the reference for determining the concentrations of unknown glycyrrhizin samples in subsequent analyses.

Table 1: Calibration standard data for Glycyrrhizin

Concentration (µg/mL)	Absorbance (Mean ± S.D)
5	0.0659 ± 0.001
15	0.1862 ± 0.0034
30	0.3752 ± 0.0059
45	0.5803 ± 0.0016
60	0.7572 ± 0.0016
75	0.9438 ± 0.0053

(n=3)

3.3 Method validation

After UV-Visible spectrophotometric method development, it becomes essential to validate the said developed method. The validation of the developed analytical method assures its sensitivity and reliability. The validation was performed according to ICH Q2 (R1) guidelines available for the validation of the analytical methods which are extensively used by the academia and the industries worldwide. There are various parameters of the analytical method validation. The set values and the limits of the parameters if obtained within the range during validation demonstrates the authentication and the reliability of the said analytical methods. For Glycyrrhizin, the developed method was validated using following parameters.

3.3.1 Linearity and Range:

The linearity of the developed UV–Visible spectrophotometric method was assessed using six pre-defined calibration standards of Glycyrrhizin over the concentration range of 5–75 µg/mL, as presented in Table No.1. Regression analysis of the calibration data demonstrated a strong linear relationship between absorbance and concentration, with correlation coefficient (r^2) values exceeding 0.999. The calibration curves constructed in triplicate exhibited uniform slopes and intercepts, indicating a consistent analytical response. The reproducibility of the calibration parameters confirmed the stability and reliability of the method across the selected concentration range, as illustrated in Figure 3 (A–C).

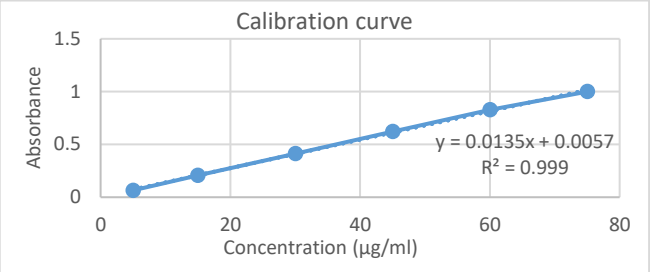

Figure 3 (A): Calibration curve replicate 1

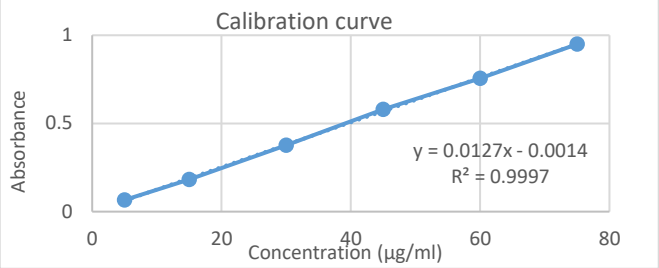

Figure 3 (B): Calibration curve replicate 2

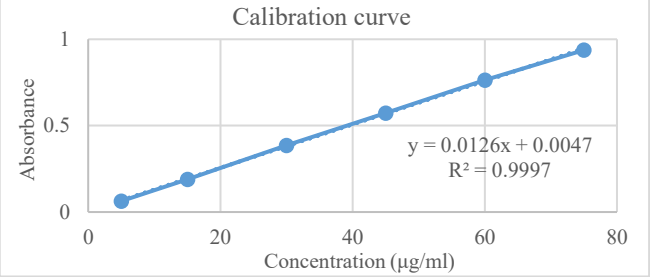

Figure 3 (c): Calibration curve replicate 3

3.3.2 Accuracy:

The closeness of agreement between the experimental value and the nominal reference value is known as accuracy. For the results to be dependable at every stage of the determination process, accuracy must be ensured over the analytical method's entire calibration range. The intra- and inter-day % difference values are shown in Table No. 2 & 3 respectively. The intra-day accuracy in terms of % difference was found to be in the range of +0.5487 to +1.8644 whereas inter-day accuracy was found to be in the range of +0.6250 to + 1.9096. Based on the obtained values, it was envisaged the proposed analytical method of is accurate

Table 2: Intra- day accuracy data of the UV method for Glycyrrhizin

Concentration Level	Nominal Concentration (µg/mL)	Mean Measured Concentration(µg/mL)	% Difference
LQC	7	7.079615	+ 1.1374
	7	7.130511	+ 1.8644
	7	7.12963	+ 1.8519
MQC	35	35.21785	+ 0.6224
	35	35.36332	+ 1.038
	35	35.39153	+ 1.1187
HQC	70	70.38408	+ 0.5487
	70	70.59612	+ 0.8516
	70	70.50705	+ 0.7244

Table 3: Inter- day accuracy data of the UV method for Glycyrrhizin

Concentration Level	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL)	% Difference
LQC	7	7.0916	+ 1.3094
	7	7.1144	+ 1.6345
	7	7.1336	+ 1.9096
MQC	35	35.2518	+ 0.7194
	35	35.3107	+ 0.8877
	35	35.4101	+ 1.1718
HQC	70	70.5555	+ 0.7936
	70	70.4613	+ 0.6589
	70	70.4375	+ 0.6250

The results indicated both intra-day and inter-day percent difference values were within acceptable limits, showing the method offers accurate and dependable measurements of Glycyrrhizin across the tested concentration range.

3.3.3 Precision:

Precision describes how closely repeated measurements agree with one another and indicates the consistency of the analytical method. In proposed study, the precision of the developed UV method for Glycyrrhizin was evaluated at three QC levels: 7 µg/mL, 35 µg/mL, and 70 µg/mL. Table No. 4 and 5 represents the % RSD values of intra-day and inter-day precision. Intra-day precision in terms of %RSD of the proposed UV Visible spectrophotometric method was found to be in the range 0.2681 to 1.3558, whereas inter-day precision of the proposed method was found to be in between 0.2581 to 1.3052. The lower values of % RSD demonstrated that the proposed analytical method of Glycyrrhizin is precise. The results confirm the developed method provides reliable and reproducible quantification of Glycyrrhizin.

Table 4: Intra-day precision data of the UV method for Glycyrrhizin

	Morning			Afternoon			Evening		
Conc. Range (µg/mL)	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
7	7.0796	0.0959	1.3558	7.1305	0.0547	0.7673	7.1296	0.0634	0.8905
35	35.2178	0.2788	0.7917	35.3633	0.2438	0.6894	35.3915	0.2924	0.8262
70	70.3840	0.1887	0.2681	70.5961	0.2944	0.4171	70.5070	0.2268	0.3217

Table 5: Inter-day precision data of the UV method for Glycyrrhizin

	Day 1			Day 2			Day 3		
Conc. Range (µg/mL)	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
7	7.0916	0.0925	1.3052	7.1144	0.0618	0.8687	7.1336	0.0690	0.9676
35	35.2518	0.2478	0.7031	35.3107	0.2752	0.7795	35.4101	0.3010	0.8501
70	70.5555	0.3374	0.4782	70.4613	0.1819	0.2581	70.4375	0.3275	0.4650

3.3.4 Robustness:

The robustness of the developed UV-Visible spectrophotometric method for Glycyrrhizin was evaluated by deliberately altering the detection wavelength by ±2 nm from the selected wavelength of maximum absorbance (λ_{max}). The variation was introduced to simulate minor wavelength shifts may occur during routine analysis due to instrument sensitivity, lamp fluctuations, or change in analyst. The absorbance values obtained under the modified conditions showed no significant deviation from the original measurements. All calculated %RSD values remained within the acceptable limit of ≤2% (Table No. 6), indicating minimal variability. The consistently low %RSD values confirmed small, intentional changes in the detection wavelength did not affect the analytical performance. The results demonstrated the proposed UV–Visible spectrophotometric method for Glycyrrhizin is robust and remains reliable under slight, deliberate variations in wavelength during routine laboratory use.

Table 6: Robustness data of the UV method for Glycyrrhizin

Concentration (µg/mL)	λ_{max} (nm)	Absorbance (Mean ± S.D.)	% RSD
7	252	0.0871 ± 0.0010	1.1609
35	252	0.4335 ± 0.0025	0.5822
70	252	0.8595 ± 0.0009	0.1109
7	254	0.0888 ± 0.0012	1.3888
35	254	0.447± 0.0020	0.4518
70	254	0.8800 ± 0.0017	0.1959

3.3.5 Limit of Quantification (LOQ) and Limit of Detection (LOD):

LOQ represents the lowest concentration can be measured with acceptable precision and accuracy. Table No. 7 displays the LOD and LOQ of the suggested UV method, which were determined to be 0.6023 and 1.82 µg/mL, respectively. The suggested method would be appropriate for assessing materials containing lower amounts of Glycyrrhizin.

Table 7: LOD & LOQ data for UV method for Glycyrrhizin.

LOD	0.6023 µg/mL
LOQ	1.82 µg/mL

3.4 Estimation of Glycyrrhizin in Marketed Formulations

The proposed UV–Visible spectrophotometric method was successfully applied for the estimation of Glycyrrhizin in three marketed Glycyrrhiza glabra tablet formulations. The assay results obtained were in close agreement with the respective label claims of Glycyrrhizin per tablet. The percentage content of Glycyrrhizin in all the analysed formulations was found to be within acceptable limits. It matched the label claims of the commercial formulations, confirming the method's accuracy and applicability for routine analysis. The results are shown in Table No. 8

Table 8: Glycyrrhizin content in commercial formulations

Sr. No.	Formulation	Brand Name & Manufacturer	Label claim of Glycyrrhiza glabra	% Assay of Glycyrrhizin (Mean ± S.D.) (n=3)
1.	Tablet	Licorice, Merlion Naturals	500 mg	104.41± 0.56
2.	Tablet	Yashtimadhu, Herb Essentials	500 mg	96.80 ± 1.03
3.	Tablet	Glycyrrhiza glabra 1X, SBL	250 mg	98.75 ± 1.20

CONCLUSION:

A simple, accurate, precise, and sensitive UV–Visible spectrophotometric method was successfully developed and validated for the estimation of Glycyrrhizin in Glycyrrhiza glabra tablet formulations. The method showed good linearity, accuracy, precision, and robustness as per ICH guidelines. The low LOD and LOQ values confirmed its sensitivity, and the assay results of marketed tablets were in close agreement with the label

claims, making the method suitable for routine quality control analysis.

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Conflict of Interest: Regarding the research authorship and/or publication of this paper, the author(s) have stated that they have no potential conflicts of interest.

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