Original Article

Swertiamarin Quantitative Analysis Using UV Spectrophotometry: A Rapid And Cost-Effective Method

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Abstract

Introduction: Ultraviolet-visible (UV-VIS) spectrophotometry is a cost effective, reliable and less time-consuming analytical technique used for the quantitative analysis, to detect the presence of impurities, chemical deterioration, and impact of stabilizers and packaging material in the pharmaceutical products. The absorbed spectrum dictates about the microscopic environments in the samples.

Objectives: The research developed and validated a simple, easy, precise, and robust quantitative analytical technique for Swertiamarin (SWMN) according to ICH criteria.

Results: The results showcased that the absorption spectrums of Swertiamarin in the UV overlay at λ_{max} 236 nm at 4 to 32 µg/ml. and linear at each concentration. Within the range of 98.5 to 104.6, the % recovery was seen. The sensitivity of the method was observed at the concentrations 0.163µg/ml (LOD) and 0.493µg/ml (LOQ).

Conclusion: Precision, repeatability, accuracy, ruggedness, and robustness were well within limitations. Quantitative UV spectrophotometry can determine a sample's (SWMN) concentration for routine analysis.

Keywords: Swertiamarin (SWMN), UV-VIS spectrophotometry, Sensitive, Precision.

INTRODUCTION

Swertiamarin (SWMN), a secoiridoid glycosidic compound of Swertia and Gentiana species[1–3], and also present in Enicostemma littorale. Apart from these, (SWMN) also presents in other plant species such as (Centaurium erythraea; Eustoma grandiflorum; Fragrea fragrans), and its presence usually responsible for numerous therapeutic actions. The content of SWMN varies in different genus and species as per the geographical locations or biological sources[2]. Mostly, HPLC and HPTLC methods were employed for the analysis and determination of SWMN in different plants and species as per previous reports[1–12].

.Chemical analysis plays an important role in the pharmaceutical industry in detecting low concentrations of hazardous chemicals which can affect human health. Therefore, the chemical analysis relies on quick, precise, and accurate modern analytical approaches for sample analysis in the pharmaceutical field[13]. Earlier reports also suggest that qualitative analysis of SWMN done by HPLC and HPTLC methods were a little bit time-consuming varying from 12-40 min.[14]. Although HPLC-HPTLC techniques are sensitive techniques. However, some of the main benefits of UV-VIS spectroscopy include relative simplicity, quick sampling speed, non-destructiveness, minimal processing time needed for data analysis, and cost-effectiveness. These features make it simple to access for analysis and getting the proof of concept work for the majority of compounds in labs, and it is primarily used for quantitative measurements[15]. UV spectrum is a graphical representation of a plot in between absorbance vs wavelength[16]. It also serves as a useful auxiliary tool for identification and structural

elucidation of pure drugs in different pharmaceutical dosage forms as per International Conference on Harmonization (ICH) guidelines-Q2(R1)[17–20].

MATERIALS AND METHODS

Materials

Swertiamarin (SWMN, CAS 17388-39-5) was purchased from Sigma Aldrich, USA. The solvents such as n-hexane-AR, petroleum ether-AR (40-60°C), ethyl acetate-AR, dichloromethane, diethyl ether-AR, chloroform-AR, methanol-AR were purchased from Qualigens Fine Chemical Pvt. Ltd, India. Digital Ultrasonicator 9L 250H/DTC PCi analytics, India was used for sonication and mixing purpose. Semi-micro weighing balance ZOHPL-OP-024 Ohaus, USA was used for weighing purpose and UV-VIS Spectrophotometer UV1800 Shimadzu, Japan was used for quantitative analysis of the drug.

Determination of absorption maxima of SWMN

A double-beam UV-VIS spectrophotometer analysed SWMN qualitatively. SWMN was scanned at 200-400 nm in methanol (MeOH) at 28µg/ml to find its absorption maxima.

Preparation of the stock solution of SWMN in MeOH

A 100 ml volumetric flask contained standard SWMN (100 mg), which had been precisely weighed. Following that, the volume created up to the mark was added before SWMN was dissolved in MeOH. A solution with a 1 mg/ml concentration was obtained by shaking the flask.

Preparation of the calibration curve for SWMN

Pipettes were used to transfer the necessary aliquots from the standard stock solution of SWMN (1 mg/ml) into 10 ml volumetric flasks. The working standard solutions were further diluted at different concentrations using methanol. As shown in **Table 2**, the analytical concentration range of SWMN was found to be 4-32µg/ml in standard solution. In order to reach concentrations of 4, 8, 12, 16, 20, 24, 28, and 32µg/mL, the volume was adjusted to the mark using methanol. As a blank, methanol was used to test the UV absorbance of each working standard solution of SWMN at its maximum wavelength (236 nm). The concentration and absorbance graphs were plotted together. Calculations were made for the regression equation and correlation coefficient.

Table 2: Standard Calibration Curve data of SWMN at 236 nm (λmax) in methanol.

Sr.no.	Concentration µg/ml	Absorbance (Mean±SD)
1	4	0.122±0.002
2	8	0.230±0.001
3	12	0.348±0.001
4	16	0.467±0.001
5	20	0.596±0.002
6	24	0.751±0.001
7	28	0.865±0.001
8	32	0.987±0.002

UV-VIS spectrophotometric method validation

The developed UV-VIS spectrophotometric method for SWMN was again validated with different parameters such as Linearity; Limit of Detection (LOD) and Limit of Quantitation (LOQ); Precision; Repeatability study (Interday and Intraday); Accuracy at different levels (80%, 100%, 120%); Ruggedness; Robustness.

Linearity

Plotting a calibration curve for SWMN at 236 nm across an analytical concentration range of 4 to 32 µg/mL. SWMN working stock solutions that had been precisely measured were transferred to separate series of 10 ml

volumetric flasks and diluted with methanol till the desired concentration. All solutions' absorbances were measured at 236 nm. Plotting concentration vs absorbance was used to create the linearity.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The lowest concentration of SWMN in the sample that can be identified but not necessarily quantitated as an accurate number is the detection limit of a certain analytical process. The lowest quantity of SWMN in the sample that can be quantitatively measured with enough precision and accuracy is the quantitation limit of a certain analytical process. The calibration curve was used to establish the LOD and LOQ of the proposed method:

$$\text{LOD} = \frac{3.3\sigma}{S} \qquad \text{LOQ} = \frac{10\sigma}{S}$$

In this equation, "S" is the slope of the calibration curve and "σ" is the standard deviation of the response (Y intercept).

i. Precision

The ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH define precision as the degree of agreement between quantity values acquired by repeated measurements of a quantity under predetermined circumstances. When evaluating the accuracy, it is necessary to use the standard deviation, variance, or coefficient of variation to mathematically describe the random error or level of dispersion of a collection of individual observations. Using a concentration of 20 µg/mL of SWMN, the intra-day and inter-day variation for SWMN determination were carried out six times on the same day and three successive days, and the percent RSD was computed.

Repeatability and Intermediate Precision

When tests are carried out in quick succession under the same circumstances (analyst, equipment, instrument, and day), they are said to be concordant when they measure the same amount again. In this study, a standard solution combination of SWMN (20μg/ml) was created and subjected to six separate analyses using the suggested methodology.

ii. Accuracy

The percentage of standard recovery was used to measure the method's accuracy. The three concentration levels of 80%, 100%, and 120% were used for recovery experiments using standard drug solution. This approach allowed for the calculation of % RSD and the known concentration of SWMN.

iii. Ruggedness

Ruggedness was assessed by doing analyses by two separate analysts, noting the corresponding percentage recovery, and reporting the findings as % RSD.

iv. Robustness

The quality of robustness is the capacity to provide accurate and exact outcomes under many circumstances. The most crucial factors were modified while the remaining parameters remained the same and operated in parallel in order to assess the robustness of the procedure. Wavelength change was the investigated parameter. The robustness study's findings, which are shown in **Table 13**, showed that the SWMN determination was unaffected by the little modification of the circumstances.

Results and Discussion

Absorption maxima of SWMN

SWMN exhibited characteristic absorption at 236 nm after scanning the solution of SWMN in methanol (MeOH) at a concentration of 28µg/ml at wavelength range of 200-400 nm. **Figure 1** shows the UV spectrum scan of SWMN in methanol. SWMN's absorption maxima was 236 nm, identical to literature (236.8 nm) [2,8] as mentioned in **Table 1**.

Table 1: Absorption maxima (λmax) of SWMN.

Name of drug	**Absorption maxima (λ_{max}) in methanol**	
	Observed in the method	**Reference**[2,20]

Swertiamarin (SWMN)	236	236.8

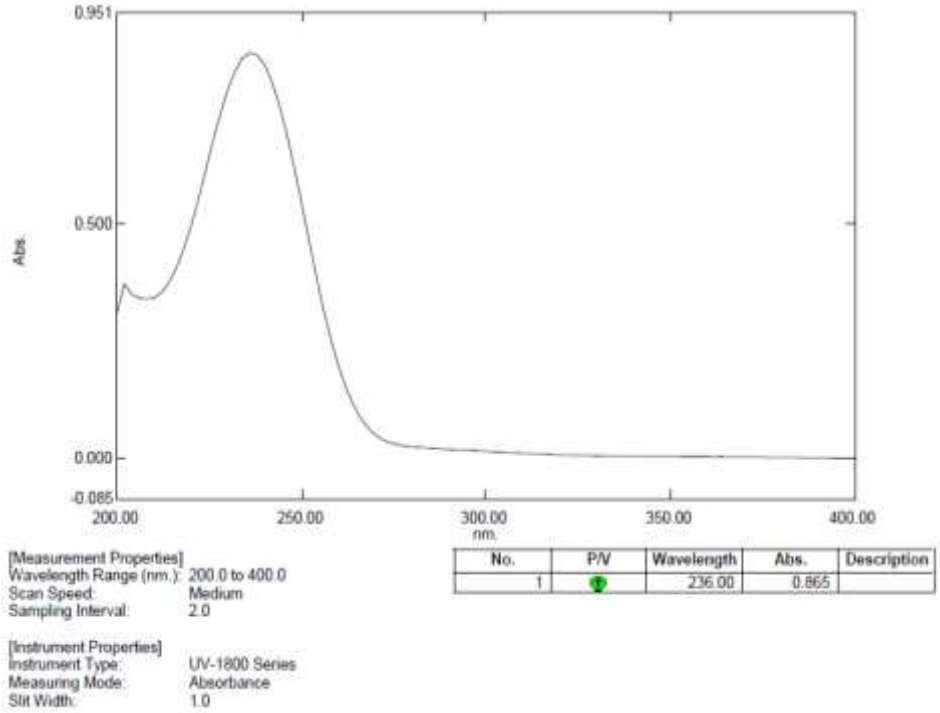

Figure 1: SWMN's ultraviolet (UV) absorption spectrum, 200–400 nm.

Standard Calibration Curve of SWMN in MeOH

The 4 to 32µg/mL SWMN concentration in methanol was used to create the SWMN calibration curve. 236 nm measured absorption. **Figure 2** depicts the findings. As seen in **Table 3**, SWMN's standard curve demonstrates strong linearity. The correlation coefficient (R2) was 0.998.

Table 3: Results of Regression Analysis of UV Method of SWMN.

Statistical Parameters	**Results**
λ_{max}	236 nm
Regression Equation	y= 0.031x-0.020
Slope (m)	0.031
Intercept (C)	0.020
Correlation Coefficient (R^2)	0.998

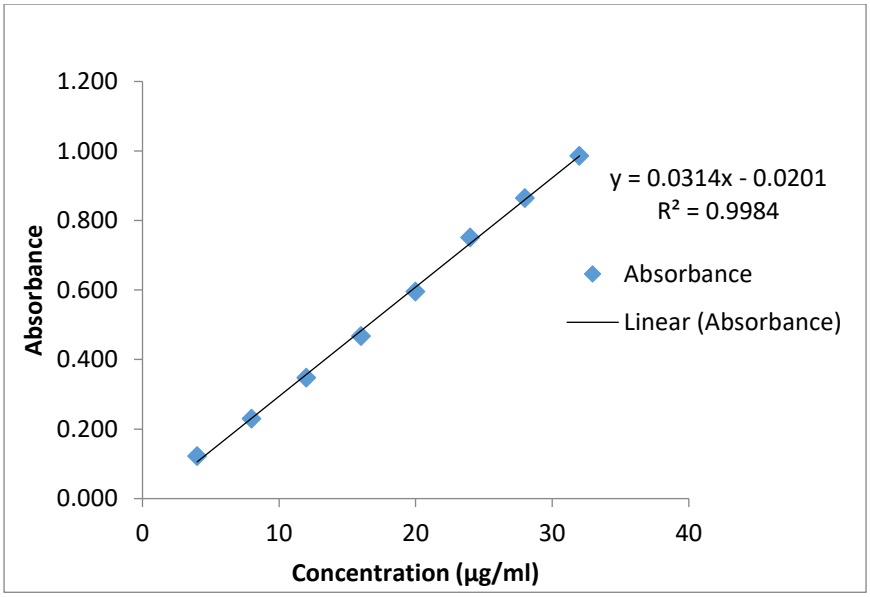

Figure 2: The SWMN standard curve.

Method validation

Linearity

As demonstrated in Table 4 and visually in **Figures 3, 4,** and **5**, SWMN's absorbance linearity at its maximum wavelength (236 nm) was seen at various concentrations (4, 8, 12, 16, 20, 24, 28, and 32 µg/mL). The overlay spectrum of SWMN from 4-32µg/mL concentration is shown in **Figure 6**. All three observations have a correlation coefficient (R2) of 0.9984, which supports the linearity of the suggested method.

Table 4: Linearity of SWMN at 236 nm (λ_{max}) in MeOH.

Sr. No.	**Concentration µg/ml**	**Linearity 1**	**Linearity 2**	**Linearity 3**
		Absorbance	**Absorbance**	**Absorbance**
1	4	0.124	0.12	0.122
2	8	0.231	0.23	0.23
3	12	0.347	0.348	0.349
4	16	0.468	0.467	0.467
5	20	0.598	0.594	0.596
6	24	0.75	0.752	0.751
7	28	0.864	0.865	0.865
8	32	0.987	0.985	0.988

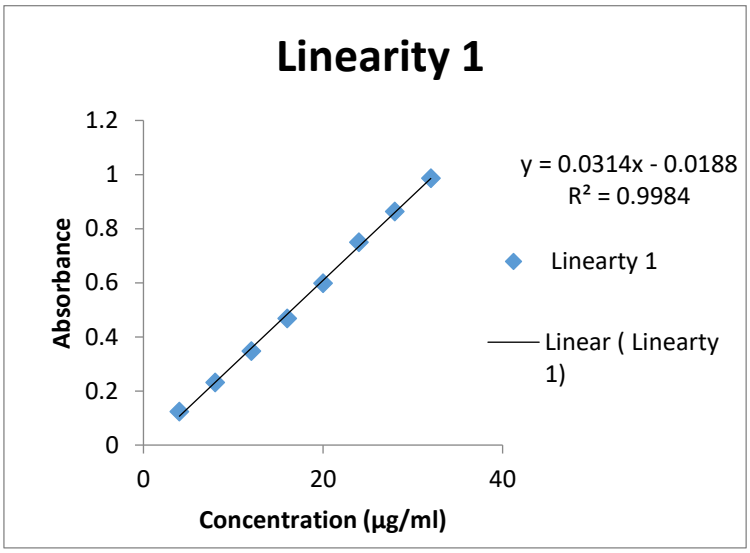

Figure 3: SWMN Linearity 1 graph.

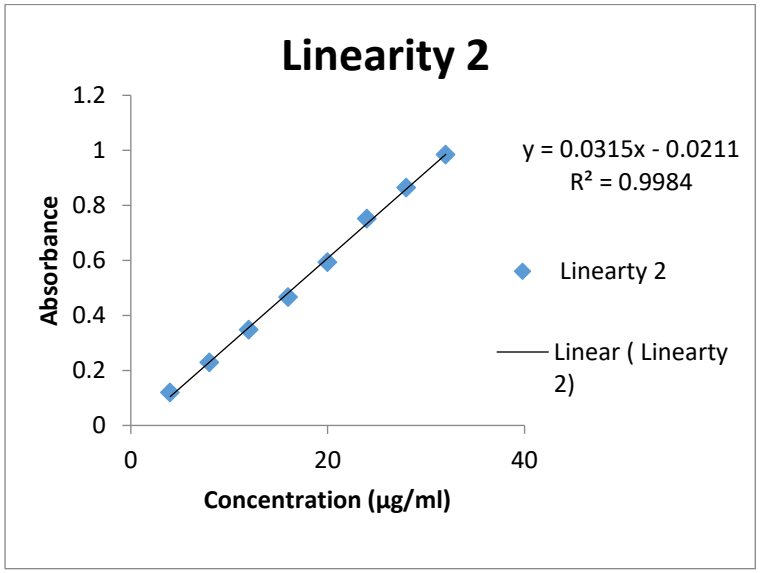

Figure 4: SWMN Linearity 2 graph.

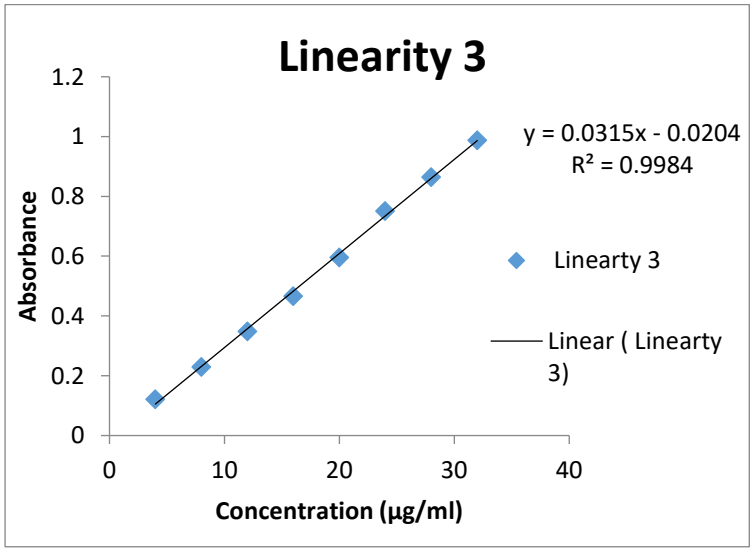

Figure 5: SWMN Linearity 3 graph.

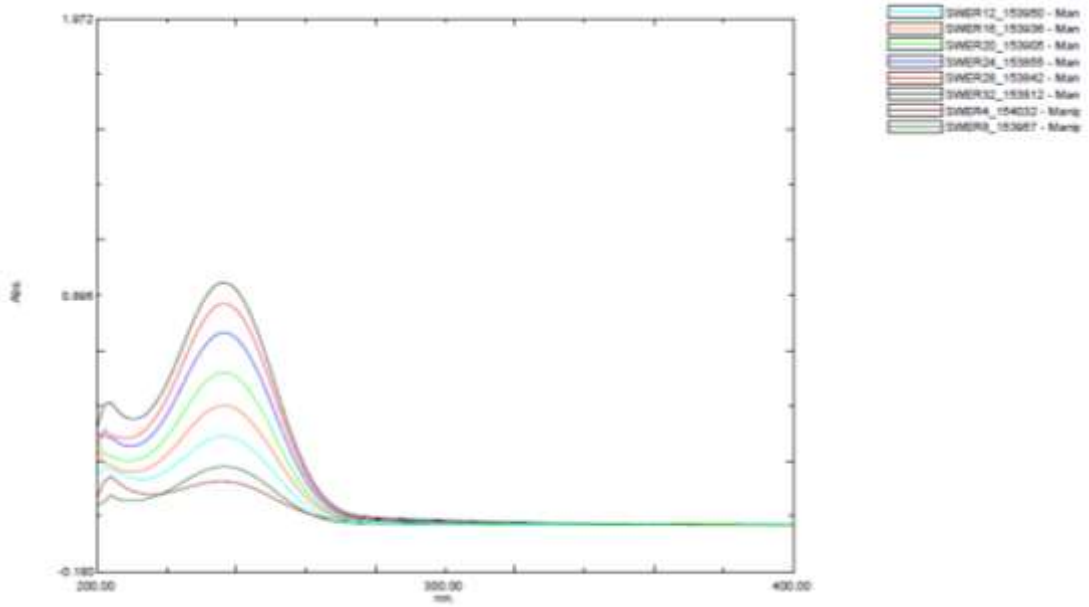

Figure 6: Overlay the SWMN UV spectrum, which ranges from 4 to 32 g/ml.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Instrumentation and data systems, however, have an impact on the LOD and LOQ values that are calculated. Due to baseline noise and drift, instrument adjustments and the use of contaminated reagents may cause significant variations in S/N ratio. By injecting progressively lower quantities of the standard solutions using the described procedures, the limit of detection (LOD) and limit of quantification (LOQ) of the UV Spectroscopic method were established. The LOD was discovered to be 0.163µg/mL and the LOQ (Table 5) was discovered to be 0.493 µg/mL, demonstrating the great sensitivity of the method.

Table 5: LOD and LOQ of SWMN.

Swertiamarin (SWMN)	
Intercept (C)	**Slope (m)**
0.018	0.031
0.021	0.031
0.02	0.031
SD of Intercept	**0.002**
Mean of Slope	**0.031**
LOD	**0.163**
LOQ	**0.493**

Precision

The repeatability, interday, and intraday variation experiments that showed the method's accuracy are shown in **Tables 6, 7,** and **8**, respectively. Calculations were made of the mean, standard deviation, and percentage of relative standard deviation. The findings demonstrate a low relative standard deviation, demonstrating the accuracy of the methods created. Low values of the %RSD were used to determine the method's precision and efficiency.

Table 6: Precision study of SWMN.

S.no.	**Drug Involved**	**Concentration**	**% Recovery**	**% Mean Recovery**	**%RSD**
1	SWMN (20µg/ml)	20.710	103.548	101.371±1.420	1.401

		20.129	100.645		
		19.935	99.677		
		20.387	101.935		
		20.065	100.323		
		20.419	102.097		

Table 7: Repeatability study (Interday) of SWMN.

S.no.	Drug Involved	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN (20µg/ml)	19.903	99.516	101.344±1.596	1.574
		20.258	101.290		
		20.710	103.548		
		19.903	99.516		
		20.452	102.258		
		20.387	101.935		

Table 8: Repeatability study (Intraday) of SWMN.

S.no.	Drug Involved	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN(20µg/ml)	19.903	99.516	100.511±0.932	0.927
		20.226	101.129		
		20.355	101.774		
		19.871	99.355		
		20.129	100.645		
		20.129	100.645		

Accuracy

Recovery tests were used to establish the method's accuracy at 80% **(Table 9),** 100% **(Table 10)** and 120% **(Table 11).** Recovery investigations were conducted three times, and for spectrophotometric estimate, the percent recovery, mean, and relative standard deviations were computed. Results show that recoveries are substantially within the 98% to 104% acceptability range, and relative standard deviation values are less than 2%, demonstrating that the method is sensitive enough to identify SWMN in samples. As a result, the procedure is reliable and useful for drug estimation.

Table 9: Accuracy Study of SWMN (Level 80%).

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN (16µg/ml)	15.677	97.984	98.589±0.605	0.613
		15.774	98.589		
		15.871	99.194		

Table 10: Accuracy Study of SWMN (Level 100%).

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN (20 µg/ml)	20.581	102.903	102.849±0.727	0.707
		20.710	103.548		

		20.419	102.097		

Table 11: Accuracy Study of SWMN (Level 120%).

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN (24 µg/ml)	25.129	104.704	104.615±0.411	0.393
		25.194	104.973		
		25.000	104.167		

Ruggedness

Changing experimental conditions examined method ruggedness. Two analysts used the identical parameters to determine mean, standard deviation, and relative standard deviation. As demonstrated in **Table 12, 13,** the spectroscopic parameters did not change, proving the spectrophotometric approaches are rugged.

Table 12: Results of Ruggedness of SWMN (Analyst 1).

S.no.	Drug Involved	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN (20 µg/ml)	20.355	101.774	102.231±1.552	1.518
		19.871	99.355		
		20.710	103.548		
		20.581	102.903		
		20.677	103.387		
		20.484	102.419		

Table 13: Results of Ruggedness of SWMN (Analyst 2).

S.no.	Drug Involved	Concentration	% Recovery	% Mean Recovery	%RSD
1	SWMN (20 µg/ml)	20.677	103.387	102.419±0.895	0.874
		20.419	102.097		
		20.645	103.226		
		20.581	102.903		
		20.226	101.129		
		20.355	101.774		

Robustness

Mean, standard deviation, and relative standard deviation were computed after changing wavelength (±5 nm). **Table 14** shows that the spectrophotometric techniques established are robust that's because the spectroscopic parameters remained unchanged.

Table 14: Robustness (Change in wavelength) for 20µg/ml.

Concentration	**Wavelength (λmax) - 236 nm in methanol**		
	232 nm	**236 nm**	**240 nm**
SWMN 20 (µg/ml)	0.618	0.642	0.621
	0.627	0.648	0.626
	0.636	0.656	0.633
	0.618	0.642	0.621
	0.626	0.631	0.622
	0.624	0.638	0.628
Mean	**0.625**	**0.643**	**0.625**

SD	**0.007**	**0.009**	**0.005**
%RSD	**1.073**	**1.329**	**0.767**

CONCLUSION

The UV spectroscopic method for Swertiamarin (SWMN) estimation was developed and validated with percentage recovery in the range of 98.589–104.615 % as according International Conference on Harmonization (ICH) Guidelines Q2 (R1) (Validation of Analytical Procedures). SWMN's absorbance linearity at λmax (236 nm) was found in varying concentrations (4, 8, 12, 16, 20, 24, 28, 32) µg/ml with a coefficient of correlation, r^2= 0.9984. In overlay UV chromatograms of SWMN from 4-32 µg/ml concentration, the absorption spectrums were linear. Because the sensitivity acceptability range was (98-104%) and the relative standard deviation was <2%, this approach was very sensitive and accurate. Data analysis of Linearity; Limit of Detection (LOD) and Limit of Quantitation (LOQ); Precision; Repeatability study (Interday and Intraday); Accuracy levels (80%, 100%, 120%); Ruggedness; Robustness demonstrated that the developed UV spectroscopic method is simple, precise, robust, and accurate, supporting it for the detection, determination, and routine analysis of SWMN in various pharmaceutical dosage forms as per ICH guidelines Q2 (R1).

CONFLICT OF INTEREST

Authors claim no conflict of interest in this research.

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