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Research Article

Development and Validation of UV Spectroscopic Method for Estimation of Symplocos Racemosa

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ABSTRACT

Background: Symplocos racemosa Roxb. Is an herbal plant grown in tropical and subtropical regions around the globe. Due to its therapeutic activity, it is being used as an herbal treatment against obesity and many other chronic and acute disease, considering these applications of the drug a UV-Spectrophotometric method was developed and validated according to ICH (International Council for Harmonization) recommendations.

Objective: To develop a quick, precise, reliable and specific UV-Spectrophotometric method validate according to ICH Q2 (R1) guidelines.

Materials and Method: Methanol was used as the solvent for method development at a wavelength of 276nm.

Results: The developed method demonstrated correlation coefficient value of 0.999and was found to be linear with a low LOQ and LOD values of 12.21 and 37 respectively with accuracy and precision %RSD (Relative standard Deviation) value less than 2.

Conclusion: A quick, accurate, simple and economical UV-Spectroscopy method was developed and validated for the estimation of Symplocos Racemosa.

Keywords: Symplocosracemosa, UV-Spectrophotometric, Obesity, Lodhra, Marketed Formulation.

INTRODUCTION

SymplocosRacemosaroxb. (SR) of family Symplococace commonly known as Lodhra, Rodhra. The leaves, bark, fruits have both medicinal and food value. SR is being used in traditional medicinal system to cure eye disease, asthma, bronchitis due to its rich antioxidant property.[1] Also SR is reported to reduce hepatocellular carcinoma, *in vitro* inhibition of lipase and used as an anti-obesity drug.[2] Obesity is a condition of accumulation of uneven fatwithin the body that impacts on human's health. The prevalence of obesity is observed to be increasing globally, thus making it amajor public health issue.[3]Overweight and obesity is being determined as the "New World Syndrome" and due to its global presence obesity is considered as the global epidemic condition by WHO (World Health Organisation).[4]

Ultraviolet-Visible spectrophotometers are the instruments that measure the ratio or function of ratio, of the intensity of two beams of light within the U.V. region.UV-Visible spectroscopy is the most widely and frequently used in pharmaceutical analysis.[5] It involves estimating the amount of visible or ultraviolet radiation a substance in solution has absorbed.[6] SR is soluble in Methanol and sparingly soluble in Ethanol, but shows insolubility in water and chloroform.

ICH (International Council for Harmonization) Q2 R1 guidelines were followed for the development and validation of an quick, precise, reliable and stable analytical method for the standard drug of SR. Validation parameters such as linearity, range, accuracy, precision and robustness were carried out as per ICH Q2 R1 preferred guidelines.[7] According to review of literature, numerous UV-Visible Spectrophotometry techniques have been developed and known to be effective for estimation of SR in plants. But the reported methods have limitations of their own, like the use of hazardous and expensive solvents and the outcomes have not been completely

verified.[8]The aim of the current study is to develop and validate a simple, accurate, quick, and reliable UV-Visible Spectroscopic analytical technique for routine estimation of SymplocosRacemosa.

MATERIAL AND METHOD

Instrumentation

For the analytical method development of SymplocosRacemosa, Shimadzu UV-1900 with scientific laboratory solutions software system was used.

Drug Sample

SymplocosRacemosa was obtained fromSindhudurg district of Maharashtra, India and was authenticated by PVP College Kavathemahankal, Sangli, Maharashtra, India.

Reagents and Chemicals

Methanol of pharmaceutical grade/ analytical grade was obtained from Arti laboratory Palghar, Mumbai and used throughout the method development and validation process.

Selection of Wavelength

SymplocosRacemosa was soluble in Methanol and hence methanol was chosen as the preferred solvent for the entire study. For working standard solution 10µg/ml of SymplocosRacemosa was observed under UV Spectrophotometer between ranges of 800nm to 200 nm.

Preparation of the Stock Solution

For preparing standard stock solution of 1000µg/ml concentration a precisely weighed 10 mg of SymplocosRacemosa was taken out in a clean and dried 10ml volumetric flask and make up to the volume with methanol. To obtain dilutions of further concentrations the aforementioned stock solution was used.[9, 10]

Estimation of Standard Calibration Curve

From the standard solutions serial dilutions of 10, 20, 30, 40 and 50 µg/ml were prepared. The absorbance was measured and a standardisation calibration curve with Concentration and absorbance was plotted on the X-axis and Y-axis respectively. The linear regression equation of the calibration curve was estimated.[11]

Method Development and Validation

International conference harmonization (ICH) Q2 (R1) validation guidelines were followed for the development and validation of the analytical method.

Validation parameters such as linearity, range, specificity, selectivity, accuracy, precision and robustness were performed.[12]

Linearity

To test the linearity parameter concentrations ranging from 10 to 50 µg/ml was used. From the standard solution of 1000 µg/ml, 1 ml is pipetted out and added methanol to make up the volume to 10ml to make a second stock solution of 100 µg/ml. Again from the second stock solution varying dilutions were made of 2, 4, 6, 8 and 10 µg/ml and make up volume with Methanol. These dilutions were assessed for linearity.[13]

Specificity and Selectivity

For calculating LOD and LOQ of the standard solution, following formulas were used with standard calibration and standard deviation of the lowest concentration range.

Limit of Detection

It is derived from the standard deviation and calibration curve of the reading of the lowest concentration range.

Limit of Detection (LOD) = $3.3 \times \frac{SD}{Slope}$

Limit of Quantification

LOQ (Limit of Quantification) is determined similar to LOD which depends on the Standard deviation and Calibration curve.

Limit of Quantification (LOQ) = $10 \times \frac{SD}{Slope}$

Precision

The degree of precision indicates how closely two measurements match. In the current study, inter-day and intra-day assays are subjected to a precision test. The intra-day precision was performed on different time intervals of the same day and for inter-day, the precision was conducted on 3 consecutive days. RSD percentage values were calculated for analytes at the determined time intervals.[14, 15]

Ruggedness

To analyse the ruggedness of the standard drug deliberate minute variations in the method was made like change in wavelength (± 2 nm), change in concentration ($\pm$ 0.2µg/ml) and change in analyte.[16]

Accuracy

For accuracy studies three distinct concentrations 50%, 100% and 150% of the sample solutions were made and mean

percentage recovery of the sample was calculated using the standardization approach. All the recovery study was calculated in triplicates from the prepared concentrations.[17]

Assay of Marketed Formulation

Different formulations containing SymplocosRacemosawere used to estimate the determination of SymplocosRacemosa, marketed SymplocosRacemosatablets and marketed SymplocosRacemosasyrup. [24] Concentration of 10µg/ml was made by diluting the formulations and the presence of Berberine was detected using the developed method.[18, 19]

RESULTS AND DISCUSSION

Method Development

With UV-1900 Spectrometer and methanol as the solvent the analytical method for SymplocosRacemosa was developed. The lambda max (λ max) of the drug was found at 276.0 nm wavelength showing the maximum absorbance. The UV spectra of SymplocosRacemosa is shown in figure 1.

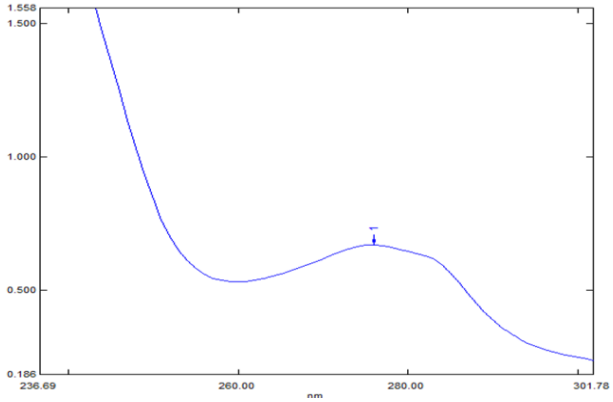

Figure 1: UV Spectra of Symplocosracemosa

Method Validation

The validation of the developed method included linearity, range, specificity, suitability, robustness accuracy and precision which was carried out as per ICH requirements.

Linearity

As per the aforementioned technique dilutions of the standard ranging from 2 to 10µg/ml analyzed for the linearity parameter. The samples showed a linear graph as depicted in figure 2 and the linearity data is shown in table 1.

Table 1: Linearity Data of Symplocosracemosa

Sr No.	Absorbance (nm)	Concentration (µg/ml)
1	0	0
2	2	0.117
3	4	0.24
4	6	0.371
5	8	0.499
6	10	0.618

R^2 = 0.999 Slope = 0.062

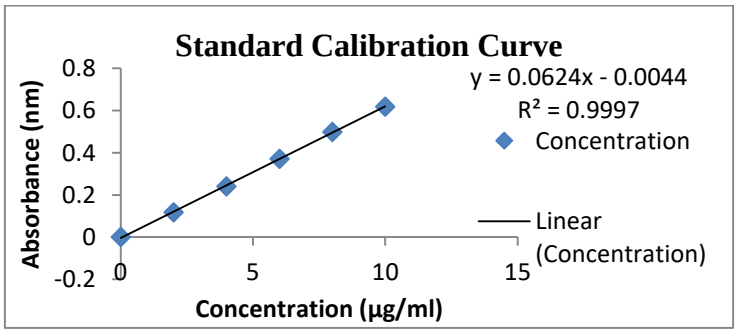

Figure 2: Linearity of Symplocosracemosa

Specificity and Selectivity

The highest absorbance of SymplocosRacemosa was observed at 276nm, indicating the developed method is specific and selective.

Limit of Detection (LOD) = $3.3 \times \frac{SD}{Slope}$

$= 12.21$

Limit of Quantification (LOQ) = $10 \times \frac{SD}{Slope}$

$= 37$

The values of LOD and LOQ were obtained to be 12.21 and 37 respectively (Table 2)

Table 2: Sensitivity Parameters and Linear Regression Data of the Developed Method

Parameters	
Concentration range (µg/ml)	2-10
R^2	0.999
Slope	0.062
Y intercept	-0.004
LOD	12.21
LOQ	37

LOD: Limit of Detection, LOQ: Limit of Quantification

Precision

To evaluate interday and intraday precision data, three distinct concentration 2, 4 and 6µg/ml of standard solution was selected and analyzed. Table 3 and table 4 depicts the interday and intraday precision data respectively.

Table 3: Interday Precision Data

Interday	**Absorbance**	**SD**	**%RSD**
Day1	0.365	0.001	0.273224
Day2	0.375	0.002646	0.703657
Day3	0.375	0.000577	0.153823

SD- Standard Deviation, RSD- Relative Standard Deviation

Table 4: Intraday Precision

Inter Day	**Absorbance**	**SD**	**%RSD**
Morning	0.369	0.002082	0.56312
Afternoon	0.369	0.002	0.542005
Evening	0.372	0.002517	0.677115

SD- Standard Deviation, RSD- Relative Standard Deviation

Accuracy

Accuracy is often estimated by measuring the samples with known concentrations and then comparing the measured values with that of the 'true' values. During recovery test a known concentration of standard was spiked into the various concentrations of 50%, 100% and 150% of the standard concentrations. The data obtained is depicted in table 5.

Table 5: Accuracy of Symplocos Racemosa

Concentration	**Absorbance**	**SD**	**%RSD**
50%	0.196	0.001	0.510204
100%	0.24	0.000577	0.241233
150%	0.301	0.001155	0.384047

SD- Standard Deviation, RSD- Relative standard deviation.

Ruggedness

To analyze the ruggedness of standard drug deliberate minute variations in the method was made like change in wavelength ($\pm$ 2 nm), change in concentration ($\pm$ 0.2 µg/ml) and change in analyte and change in analyte. The robustness results obtained are reported in table 6.

Table 6: Robustness Data of Symplocos Racemosa

Robustness	Parameters	Absorbance	SD	%RSD
Change of Wavelength	274nm	0.359	0.001528	0.4251
	278nm	0.362	0.002517	0.695837
Change of Concentration	5.8µg/ml	0.360	0.001528	0.42392
	6.2 µg/ml	0.424	0.001	0.235849
Change of Analyte	Analyte 1	0.364	0.001528	0.419266
	Analyte 2	0.366	0.000577	0.15789

SD: Standard Deviation, RSD: Relative Standard Deviation.

Assay of Marketed Formulation

The assay data for the marketed formulations are represented in table 7. The proposed model has a significant correlation to the mean response

Table 7: Estimation of Symplocos Racemosa in Marketed Formulations

Formulation	Absorbance
Symplocos Racemosatablets	0.407
Symplocos Racemosasyrup	0.538

CONCLUSION

The developed method for estimation of Symplocos Racemosa is a simple, quick, reliable and accurate as per the validation parameters performed according to ICH Q2 R1 guidelines pertaining to development of analytical methodology. The developed method showed great, linearity, precision, accuracy and specificity with low LOD and LOQ values. The developed analytical method meets the specific acceptance criteria and it can be used for the estimation of SymplocosRacemosa in plants.The results showed that the excellent scope of the UV-method for determining SymplocosRacemosain marketed formulations and they were well within acceptance criteria.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Abbrevations

SR: SymplocosRacemosa; **ICH: International** Council for Harmonization;**LOD:** Limit of Detection; **LOQ:** Limit of Quantification;**µg:** Microgram; **nm**: nanometre; **WHO:** World Health Organisation; **UV:** Ultraviolet-Visible; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation.

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