RESEARCH ARTICLE

Development and Validation of the UV-Spectrophotometric Method for Determination of *Vigna radiata* Seed Extract

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Received: 23/10/2024 **Accepted: 30/12/2024** **Published: 15/01/2025**

Abstract

Aim: The main goal was to develop and validate a sensitive, simple, accurate, precise, ruggedness and cost-effective UV spectrophotometric method for estimating *Vigna radiata* seed extract (VR) in prepared pharmaceutical formulations of smart lipid nanoparticles.

Methodology: The standard solution was prepared in water, and a calibration curve was constructed after measuring the absorbance. The different analytical performance parameters, such as linearity, range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness, were determined according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines.

Results: *Vigna radiata* seed extract showed maximum absorption at a wavelength of 281 nm**.** Beer-Lambert's law was obeyed in the concentration range of 25-400 µg/ml with a correlation coefficient (R^2) of 0.9968. The limit of detection and limit of quantification was found to be 16.03 µg/ml and 48.57 µg/ml respectively. The precision and repeatability scores were all within acceptable limits. The recovery was found to be between 99.33% and 100.2% The precision and repeatability values were within a 2% tolerance range. The extract was found to have a purity of 99.33%

Conclusion: The study demonstrated that the developed procedure was accurate, precise and reproducible while being easy, environmentally friendly, repeatable, and cost-effective, and it can be used for quantification of *VR* seed extract in pharmaceutical dosage forms.

Keywords: *Vigna radiata* seed extract, Beer's law, UV spectrophotometry, Method development, Validation.

Journal of Applied Pharmaceutical Sciences and Research, (2024); DOI: 10.31069/japsr.v7i4.04

Introduction

Mung bean seeds (*V. radiata* (L.) R. Wilczek) belonging to the family Fabaceae, are known as the 'Green Pearl' of Asia. It is a widely used traditional cuisine over the world.[1] The mung bean is also known as mash, golden gram, green gram, and mung beans. This species ranges from little plants to big tropical canopy trees. This plant species can be found in humid tropics, temperate zones, highlands, dry areas, and lowlands.[2] This is the optimal source of protein, minerals, and vitamins. Previously, Mung beans were called Phaseolus aureus or *Phaseolus radiata*. In the 1970s, mung beans were reclassified as *Vigna* rather than Phaseolus. The mung bean plant offers numerous health benefits, including disease prevention.[3] Mung bean seeds have traditionally been used to treat alcoholism. It is commonly used for antipyretic, antiscorbutic, diuretic, antidote, antihypertensive, and anticancer purposes. An Oriental herbalist advised it for pain, fever, elevated blood pressure and inflammation. Mung bean seeds, whether raw or cooked, are effective in treating polyneuritis galinarum. In India, mung bean seeds are used to treat cough, paralysis, fever, and rheumatism. They are used to treat piles and liver ailments and as a hot tonic. Mung bean root has narcotic properties and is used to treat bone aches.[4,5]

Mung bean seeds exhibit anticancer properties,[6] antihyperlipidemic,[7,8] antihypertensive,[9] antidiabetic,[10] antioxidant,[11,12] antiviral,[13] antifungal, and antibacterial.[14] Mung beans can help prevent obesity and disorders caused by high-fat diets[15] and are beneficial for liver disease.[16] Research suggests that it can improve physical strength, treat rheumatism, and prevent Alzheimer's disease.[17,18] The absence of a detailed pharmacognostic study on Mung seeds (*V. radiata* (L.) R. Wilczek) in the literature led to the current analysis.

This research aims to address this need by presenting the development and validation of a UV-spectrophotometric method for the quantification of VR seed extract. By establishing a validated UV-spectrophotometric method for VR quantification, this research seeks to provide a valuable tool for quality control and standardization of VR-containing products in the pharmaceutical and herbal industries. Furthermore, this method can facilitate pharmacokinetic studies, formulation development, and therapeutic monitoring of VR-based interventions, contributing to the

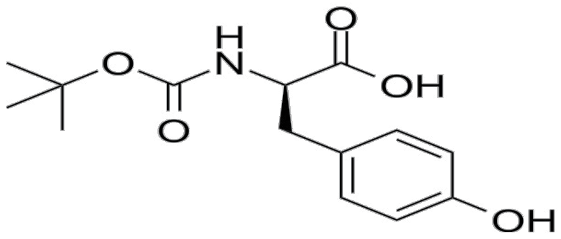

Figure 1: Chemical structure of VR

advancement of research and applications in the field of natural product pharmacology and phytotherapy Figure 1.[19]

Material and Methods

Materials

For analytical method development, a Shimadzu UV-1900 with the scientific laboratory solutions software system and a Shimadzu UV-1800 with UV Probe software system were utilized. *Vigna radiata* seed extract was procured from Ambe NS Agro Products Pvt. Ltd., Ghaziabad, Uttar. Pradesh, India, along with its certificate of analysis. Distilled water was used as a solvent for all experiments.

Selection of wavelength for analysis of *Vigna radiata*

A working standard solution of VR (10 µg/ml) was prepared in water and scanned using a UV spectrophotometer between 355 nm and 220 nm, revealing the highest absorption at 281nm.

Preparation of standard stock solution

Accurately weighed 10 mg of VR was transferred to a 100 ml volumetric flask. The volume was adjusted with water up to the mark resulting in a final concentration of 100 µg/ml. This solution served as a standard stock solution. Further dilutions were made using this standard stock solution.[20,21]

Preparation of calibration curve

Serial dilutions of 25,50,100,150,200,250,300,350 and 400 µg/ml were made from the standard stock solution. The absorbance of the solution was measured at 281nm, a standardization curve was produced with concentration on the X-axis and absorbance on the Y-axis, and a linear regression equation was calculated.[20,21]

Method development and validation

In water, VR seed extract was shown to be soluble. As a result, this solvent was utilized to determine the detection wavelength and standard dealing concentration. The International Conference on Harmonization (ICH) has issued validation guidelines for analytical techniques, which characterize this method as characteristic performance verified through laboratory research. The developed technique was validated by ICH recommendations on validation of analytical procedures: text and methodology, Q2(R1).[19]

Specificity and selectivity

The blank solution was scanned between 200nm-600nm to see if there was any substantial absorbance at the wavelength where VR absorbs the most. Water was used as a blank. An overlay spectrum of VR was also created to ensure that the absorbance observed at the wavelength of interest (281 nm) in the sample solution is solely due to the presence of VR.[20]

Linearity

Standard solutions were generated with concentrations ranging from 1-20µg/mL. The dilutions of the stock solution were made by diluting the necessary aliquot with the solvent. The absorbance of each solution was measured at 281 nm with the same solvent system as the blank. A calibration curve was created by plotting concentration on the x-axis and absorbance on the y-axis, and linearity was calculated using a regression equation. The experiment was repeated three times.[22]

Precision

The precision of the method was assessed through intraday and interday variability studies. Intraday precision was evaluated by analyzing Vigna radiata (VR) seed extract solutions at concentrations of 25, 200, and 400 µg/mL, three times within the same day. For interday precision, the same concentrations were analyzed once daily over three consecutive days. The relative standard deviation (RSD) for each concentration was calculated to determine the interday precision.[22,23]

Accuracy

Accuracy was demonstrated through recovery studies, where the percent mean recovery of the sample was computed using a standardization approach at three concentration levels: 50%, 100%, and 150% of the sample solutions. Dilutions were prepared from the stock solution as needed. For each level, three replicates were prepared, and recovery studies were performed to validate the method.[23,24]

Ruggedness

The ruggedness of the proposed method was evaluated for a 200µg/ml concentration of VR seed extract by analysing aliquots from a homogenous sample batch. The analysis was conducted by two different analysts under identical operational and environmental conditions to ensure the consistency and reliability of the method.[23,25]

Robustness

The robustness of the proposed method was assessed for a 10µg/ml concentration of VR seed extract by varying the temperature i.e. 25±10°C and at different wavelengths i.e.

281 ± 1nm. [23,26]

Sensitivity

The sensitivity of the proposed method for measuring VR seed extract was estimated in terms of the limit of quantification (LoQ) and limit of detection (LoD). The LoQ and LoD were calculated using the equations:[27,28]

$$\text{Limit of Detection}\left(\text{LOD}\right) = 3.3\times\frac{\sigma}{S}$$

$$\text{Limit of Quantification}\left(\text{LOQ}\right) = 10\times\frac{\sigma}{S}$$

Where σ = the standard deviation of the response; S = the slope of the regression line.

Results And Discussion

Method development

Using the UV-1800 equipment and water as a solvent, a UV-spectrophotometric technique was devised.

Method validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment. The developed method was validated in terms of specificity, selectivity, linear range, precision, robustness, ruggedness, and reproducibility.

Specificity and Selectivity

The UV spectrum of VR seed extract exhibited a maximum absorbance (λmax) at 281nm, demonstrating the method's specificity and selectivity (Figure 2). The spectra of the blank solution indicated no absorbance at the wavelength corresponding to the VR seed extract, further confirming the method's selectivity. Overlay spectra analysis demonstrated consistent λmax values across the concentration range of 25–400 µg/mL, with no significant spectral variations observed (Figure 3). These results establish the UV spectrophotometric method as both specific and selective for the analysis of VR seed extract, ensuring reliable detection across the tested concentration range.

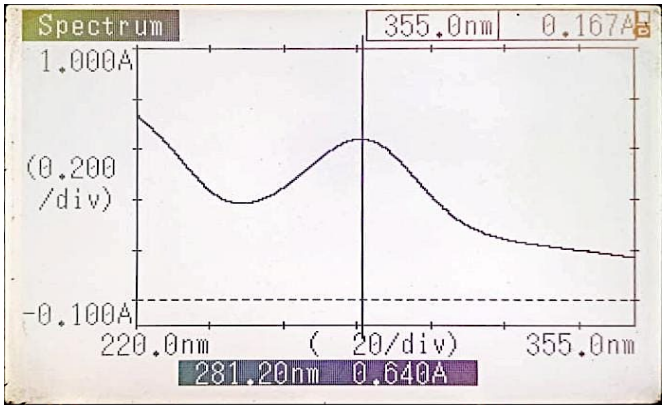

Figure 2: UV Absorption spectra of Vigna radiata at 281 nm

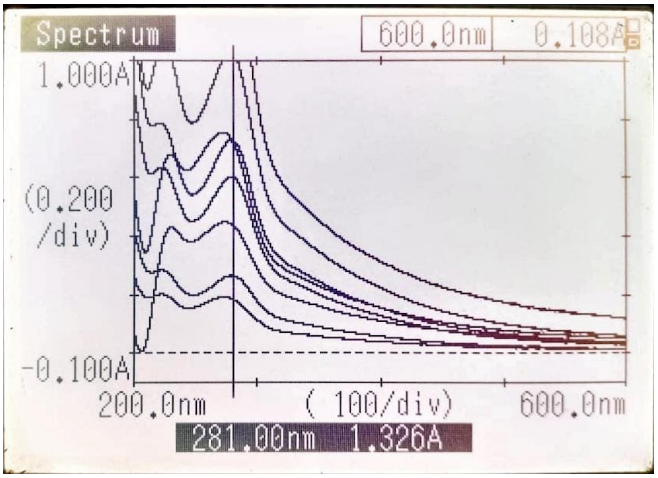

Figure 3: Overlay UV spectra of Vigna radiata in water showing maximum absorbance at 281nm

Linearity

Dilutions spanning the concentration range of 25-400µg/ml were analysed, and absorbance values were noted in triplicate. The calibration curve was plotted, and linear regression data demonstrated a strong linear relationship across the concentration range tested. The mean absorbance range (n=3) was found to range from 0.053 ± 0.001 to 0.944 ±0.003, with RSD values below 2 %, as shown in Table 1. The linear regression equation was determined assay = 0.0024x + 0.0085 with a coefficient of correlation value of $R^2 = 0.9968$ (Figure 4).

This robust linear relationship confirms that the method is highly effective in accurately quantifying *Vigna radiata* within the specified concentration range. The high R^2 value further validates the method›s linearity, indicating an excellent fit of the model to the experimental data and ensuring reliability for quantitative analysis.

Table 1: Linearity data of *Vigna radiata*

Concentration (µg/ml)	*Mean absorbance at 281 nm ± SD*	*% RSD*	*Regressed Absorbance*
25	0.053± 0.001	1.886	0.054
50	0.125 ± 0.002	1.218	0.126
100	0.272 ±0.002	0.766	0.272
150	0.375 ±0.001	0.153	0.377
200	0.462 ±0.002	0.432	0.46
250	0.592 ±0.002	0.257	0.59
300	0.721 ±0.001	0.080	0.722
350	0.863 ±0.001	0.115	0.865
400	0.944 ±0.003	0.280	0.945

*The data is expressed as mean± SD, n=3.

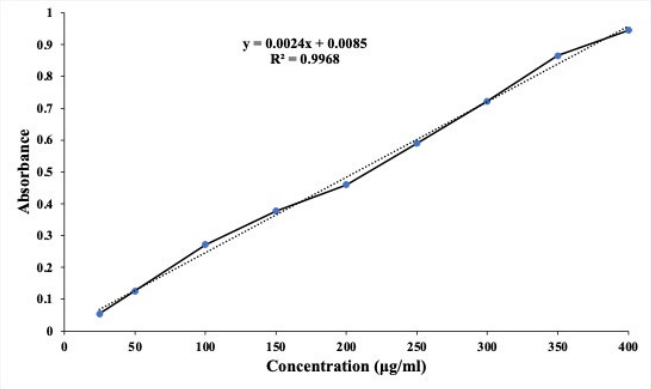

Figure 4: Calibration Curve of VR Seed extract

Precision

Following the procedure, three replicates of solutions containing *Vigna radiata* at concentrations 25µg/ml, 200µg/ml, and 400µg/ml were prepared. The absorbance of each solution was measured at 281nm to assess system precision. The %RSD was calculated, and it was found to be less than 2% as given in Table 2. To assess interday precision, three replicates of solutions containing *Vigna radiata* seed extract at concentrations of 25µg/ml, 200 µg/ml, and 400 µg/ml were prepared. The absorbance of each solution was measured on three consecutive days to evaluate variability over time. %RSD was calculated for each concentration across the three days. The %RSD values obtained for interday precision (Table 3) were found to be less than 2% for all tested concentrations. This indicates excellent reproducibility of the measurements over multiple days, demonstrating the method's reliability and stability in different experimental sessions. The low %RSD values signify minimal variability between the replicates measured on different days, further validating the precision of the UV method for VR analysis.

Accuracy

The accuracy of the analytical method was evaluated through recovery studies conducted at three levels: 50%, 100%, and 150% of the expected sample concentrations. At each level, sample solutions were prepared and analyzed using the standardization approach. The measured amount of VR in the samples was consistent with the label claim of the formulation. The percent mean recovery was calculated to be 99.33%, with recovery values ranging between 99.33% and 100.2% (Table 4), demonstrating excellent accuracy. While the results indicate the method's ability to accurately quantify VR in the sample solutions, it is important to note that the %RSD exceeded 2%, indicating some variability in the recovery values. Despite this, the overall accuracy of the method validates its reliability for the precise and accurate determination of VR content in pharmaceutical formulations. Further optimization may help reduce variability and enhance method robustness for broader applications.

Table 2: Intraday Precision Data

Concentration (µg/ml)	*Abs 1*	*Abs 2*	*Abs 3*	*Mean Absorbance ± SD*	*%RSD*
25	0.054	0.056	0.055	0.055 ± 0.001	1.818
200	0.46	0.45	0.465	0.458 ± 0.007	1.666
400	0.945	0.942	0.948	0.945 ± 0.003	0.317

*The data is expressed as mean± SD, n=3.

Ruggedness

The ruggedness of the developed was assessed by involving independent analysts to check the repeatability. The % RSD for repeatability was found to be less than 2% (Table 5), indicating that the method is rugged. The consistency of results obtained across different analysts suggests that the method is not significantly affected by minor variations in experimental conditions or personnel. This indicates the method's ruggedness and suitability for routine analysis, providing reliable and consistent results for the quantification of VR.

Robustness

The robustness of the method was assessed by subjecting it to variations in temperature and wavelength. Temperature changes were introduced by analyzing the sample solutions at different temperatures, while wavelength variation was achieved by measuring absorbance at wavelengths slightly different from the methods specified wavelength.

Despite these intentional variations, the method exhibited consistent and reproducible results. The %RSD values for measurements conducted at different temperatures and wavelengths were found to be within acceptable limits, typically less than 2% (Table 6). This indicates that the method is robust and capable of providing reliable results even under slightly altered experimental conditions.

Table 3: Interday Precision Data

Concentration (µg/ml)	*Abs 1*	*Abs 2*	*Abs 3*	*Mean Absorbance ± SD*	*%RSD*
25	0.054	0.055	0.056	0.055 ± 0.001	1.818
	0.052	0.054	0.053	0.053 ± 0.001	1.886
	0.051	0.052	0.051	0.051 ± 0.0005	1.124
200	0.46	0.458	0.459	0.459 ± 0.001	0.217
	0.45	0.46	0.462	0.457 ± 0.006	1.405
	0.466	0.457	0.449	0.457 ± 0.008	1.859
400	0.945	0.936	0.924	0.935 ± 0.010	1.126
	0.95	0.946	0.932	0.942 ± 0.009	1.002
	0.925	0.934	0.938	0.932 ± 0.006	0.714

*The data is expressed as mean± SD, n=3.

Table 4: Accuracy and Recovery

% of standard spiked to the sample	*Sample (µg/ml)*	*Amount (µg)*		*% drug recovered*	*% RSD*
		Total including spiked sample	*Spiked sample determined SD (n=3)*		
50	200	300	298±1	99.33	0.335
100	200	400	398.67±0.58	99.66	0.144
150	200	500	501±2	100.2	0.399

*The data is expressed as mean± SD, n=3.

Sensitivity

The limits of detection (LOD) and quantification (LOQ) for VR seed extract were determined to be 16.03 µg/ml and 48.57 µg/ml, respectively. These values represent the lowest concentration of VR that can be reliably detected and quantified with acceptable accuracy and precision using the method.

The low LOD and LOQ values indicate that the method is highly sensitive and capable of detecting and quantifying VR at trace levels in complex sample matrices. This demonstrates the method's suitability for applications requiring the detection of VR at low concentrations, such as in pharmacokinetic studies or quality control analyses of herbal products.

Table 5: Ruggedness Study by Two Analysts

	Analyst 1	*Analyst 2*
Concentration (200 µg/ml)	0.46 0.465 0.464 0.463 0.465 0.462	0.461 0.459 0.463 0.464 0.464 0.465
Mean Absorbance	0.463	0.462
S.D	0.001	0.002
%RSD	0.419	0.486

*The data is expressed as mean± SD, n=6.

Table 6: Robustness study of the proposed method

Condition	*Parameter*	*Absorbance*	*Mean* ± SD	*%RSD*
Change in	282 nm	0.639	0.641 ± 0.003	0.501
wavelength	281 nm	0.64		
	280 nm	0.645		
Change in	15°C	0.64	0.645 ±0.005	0.794
temperature	25°C	0.647		
	35°C	0.65		

*The data is expressed as mean± SD, n=3.

Conclusion

The developed and validated UV-spectrophotometric method for the determination of VR seed extract proved to be simple, precise, accurate, and robust. This method demonstrated reliable performance across various parameters, including precision, accuracy, sensitivity, ruggedness, and robustness, making it suitable for routine analysis in quality control laboratories. Its simplicity and cost-effectiveness further enhance its practical applicability, particularly in settings with limited access to sophisticated analytical equipment.

In the future, this method could be further explored for its application in analysing VR seed extract in complex formulations, such as pharmaceuticals, cosmeceuticals, nutraceuticals, or food supplements. Additionally, coupling this method with advanced analytical techniques like HPLC or LC-MS could enhance its sensitivity and selectivity, enabling the detection of trace phytochemical components. Studies on its scalability for industrial applications and its integration into regulatory frameworks could further establish this method as a standard for *Vigna radiata* analysis. Additionally, exploring the method's scalability for industrial applications and its alignment with regulatory guidelines could establish it as a gold standard for the quality control of *Vigna radiata*-based products.

Conflict of Interest

The author declares no conflict of interest, financial or otherwise.

Acknowledgements

The authors are thankful to the I.T.S College of Pharmacy, Murad Nagar, Ghaziabad for providing the infrastructure and chemicals for the research.

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How to cite this article: Singh P, Chauhan I, Verma M, Yasir M, Majhi S. Development and Validation of the UV-Spectrophotometric Method for Determination of *Vigna radiata* Seed Extract. Journal of Applied Pharmaceutical Sciences and Research. 2024; 7(4):21-27 Doi : 10.31069/japsr.v7i4.04