RESEARCH ARTICLE

Development and Validation of UV-Spectrophotometric Method for Berberine Quantification

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Received: 10/04/2024 **Accepted: 03/05/2024** **Published: 15/07/2024**

Abstract

Aim: The present work aimed to develop and validate a sensitive, simple, accurate, precise, ruggedness and cost-effective ultraviolet (UV) spectrophotometric method for the estimation of Berberine (BBR) in prepared pharmaceutical formulations of smart lipid nanoparticles.

Methodology: The standard solution was prepared in methanol and a calibration curve was constructed after measuring 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 International Conference on Harmonization (ICH) Q2 (R1) guidelines.

Results: BBR showed a maximum absorption (λmax) at a wavelength of 418 nm. Beer-Lambert's law was obeyed in the concentration range of 1 to 15 µg/mL with a correlation coefficient (R2) of 0.9922. The limit of detection and limit of quantification was found to be 1.565 and 4.742 µg/mL, respectively. The precision and repeatability scores were all within acceptable limits. The recovery was found to be between 99.64 and 100.39%. The precision and repeatability values were within a 2% tolerance range. BBR was found to have a purity of 99.64%.

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 BBR in pharmaceutical dosage forms.

Keywords: Berberine, Beer's law, UV spectrophotometry, Method development, Validation.

Journal of Applied Pharmaceutical Sciences and Research, (2024); DOI: 10.31069/japsr.v7i2.05

Introduction

Berberine (BBR) is a phytochemical present in a variety of plants, mostly belonging to the Berberidaceae, Ranunculaceae and Papaveraceae families.[1] It has a bright yellow color, bitter flavor and is chemically known as 9,10-dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-g]isoquino[3,2-a]isoquinolin-7-ium (Figure 1).[2] BBR is an isoquinoline alkaloid found in several medicinal plants and possesses a molecular weight of 353.36 with molecular formula $C_{20}H_{19}NO_5$. Plants including Guduchi, *Berberis vulgaris*, goldenseal, goldthread, oregon grape, and rosid dicot genera have this component.[1,3] BBR alkaloids have a wide range of pharmacological activities, such as bactericide, antiviral, antibacterial, anti-inflammatory blood pressure reduction, hypoglycaemic, medicine, and tumor metastatic effects.[3]

Various methods for the analysis of BBR have been explored, as detailed in Table 1. Each of these methods offers distinct advantages and limitations in terms of sensitivity, selectivity, and practical applicability. Among the analytical techniques available, UV-spectrophotometry stands out as a simple, cost-effective, and widely used method for quantitative analysis. UV-spectrophotometric methods rely on the measurement of absorbance of UV or visible light by a compound in solution, allowing for the determination of its concentration based on the Beer-Lambert law. In the context of BBR analysis, UV-spectrophotometric methods offer several advantages, including high sensitivity, broad applicability, and minimal sample preparation requirements. However, the development of a robust UV-spectrophotometric method for BBR quantification requires careful optimization of experimental parameters and validation to ensure accuracy, precision, specificity, and linearity.[4]

This research aims to address this need by presenting the development and validation of a UV-spectrophotometric method for the quantification of BBR extract. By establishing a validated UV-spectrophotometric method for BBR quantification, this research seeks to provide a valuable tool for quality control and standardization of BBR-containing products in the pharmaceutical and herbal industries.

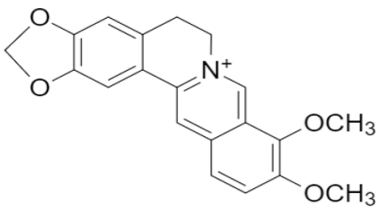

Figure 1: Chemical structure of berberine

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Furthermore, this method can facilitate pharmacokinetic studies, formulation development, and therapeutic monitoring of BBR-based interventions, contributing to the advancement of research and applications in the field of natural product pharmacology and phytotherapy.[4]

Material and Methods

Instrumentation

For analytical method development of BBR, Shimadzu UV-1900 with scientific laboratory solutions software system and Shimadzu UV-1800 with UV Probe software system were utilized.

Drug and Reagents

BBR extract 97% was procured from Ambe NS Agro Products Pvt. Ltd., Ghaziabad, Uttar. Pradesh, India. Methanol was purchased from Central Drug House (P) Ltd., Delhi, India.

Selection of Wavelength

A working standard solution of BBR (10 µg/mL) was prepared in methanol and scanned using a UV spectrophotometer between 600 and 200 nm, revealing the highest absorption at 418 nm.

Preparation of Standard Stock Solution

Accurately weighed 10 mg of BBR was transferred to a 100 mL volumetric flask. The volume was adjusted with methanol 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.[3,5]

Preparation of Calibration Curve

Serial dilutions of 1, 2, 5, 7, 10, 12, 14, and 15 µg/mL were made from the standard stock solution. The absorbance of the solution was measured at 418 nm, a standardization curve was produced with concentration on the X-axis and absorbance on the Y-axis, and a linear regression equation was calculated.[3,5]

Method Development and Validation

In methanol, BBR 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.[4]

Specificity and Selectivity

A blank solution using the solvent (e.g., methanol) was prepared without BBR. The blank solution was scanned over the same wavelength range and observed if there was any significant absorbance at the wavelength corresponding to the maximum absorption of BBR. An overlay spectra of BBR was also made to ensure that absorbance observed at the wavelength of interest (λ_{max}= 418 nm) in the sample solution is due to the presence of BBR only.[3]

Linearity

The standard solutions were prepared in the range of 1 to 20 µg/mL. The dilutions of the stock solution were prepared by diluting the required aliquot with the solvent system. The absorbance of each solution was measured at 418 nm using the same solvent system as the blank. A calibration curve was constructed by plotting concentration on the x-axis and absorbance on the y-axis and linearity was determined using a regression equation. This experiment was repeated three times.[6]

Precision

The precision of the method was assessed through intraday and interday variations. For intraday precision, 2, 10, and 15 µg/mL solutions of BBR were analyzed three times within the same day. Interday precision was evaluated by analyzing 2, 10, and 15 µg/mL solutions of BBR daily for three consecutive days over a week. The relative standard deviation (RSD) was calculated for each concentration across the three days to assess interday precision.[6,7]

Accuracy

Accuracy was confirmed by doing recovery experiments in which the percent mean recovery of the sample was calculated using a standardization approach at three distinct levels: 50, 100, and 150% of the sample solutions. Any dilutions are made from the above solution. Three replicates of the concentration solution were prepared for each level, and a recovery study was conducted.[13]

Ruggedness

The ruggedness of the proposed method is determined for 10 µg/mL concentration of BBR by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions.[14]

Robustness

The robustness of the proposed method is determined for 10 µg/mL concentration of BBR by changing the temperature, i.e., 25 ± 10°C and at different wavelengths, i.e., 418 ± 1nm. [15]

Sensitivity

The sensitivity of measurements of BBR by the use of the proposed method was estimated in terms of the limit of quantification (LoQ) and limit of detection (LoD). The LoQ and LoD were calculated using the equations:[16-17]

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

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

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

Table 1: A brief review of previously developed analytical methods for BBR

S.No	Method	Detection	Linearity range/ scan range	LoD	LoQ	Equation/Linear Regression Coefficient	Ref.
1	UV	422 nm	10–50 µg/mL	2.81	8.54 µg/mL	R^2= 0.9996	3
2	HPLC-UV	346 nm	569–1053 µg/mL	-	-	y = 2763 × –29659 ,r^2 = 0.9942	5
3	RP-HPLC	344 nm	2–12 µg/mL	0.488 µg/mL	1.478 µg/mL	y = 57.86.x+0.407	6
4	RP-HPLC	288 nm	10–30 µg/mL	0.19 µg/mL	0.59 µg/mL	Y=495432.7x+110737 (R^2= 0.999)	7
5	RP-HPLC	266 nm	20–640 mg/mL	0.8	1.7	y=68734x2054, r^2=0.999	8
6	HPTLC	366 nm	120–360 ng /spot	40 ng	120 ng	y= 63.647x+7728.450	9
7	HPLC–PDA	230 & 240 nm	2.50–25.00 µg/mL	0.131–0.296 µg/mL	0.398–0.898 µg/mL	$R^2 \geq 0.9994$	10
8	Electroc hemical	8.0×10^{-7}mol /L	30–250 mV/s	$8.0x10^{-7}$ mol/l	-	(10^{-7}A) = 2.6688+ 0.3784 C (10^{-5} mol/L)	11
9	Spectrofl uorimetric	excitation wavelength =360 nm emission wavelength =465 nm	1.0×10^{-6} to 8.0×10^{-5} mol L^{-1}	15.76 ng/mL	47.78 ng/mL	-	12

Results And Discussion

Method Development

Using the UV-1800 equipment and methanol 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 BBR exhibited maximum absorbance (λ_{max}) at 418 nm, indicating the specificity and selectivity of the method (Figure 2). In a previous study, Tavade *et al.* reported the maximum absorption wavelength of BBR hydrochloride at 422 nm.[3] The spectra of the blank solution revealed no absorbance at the wavelength of BBR ensuring the method to be selective. Notably, no significant change in the λ_{max} of BBR was observed across the tested concentration range of 1 to 15 µg/mL, as evidenced by overlay spectra analysis (Figure 3). The consistent λmax and absence of spectral changes within the tested concentration range affirm the specificity and selectivity of the UV method for BBR analysis.

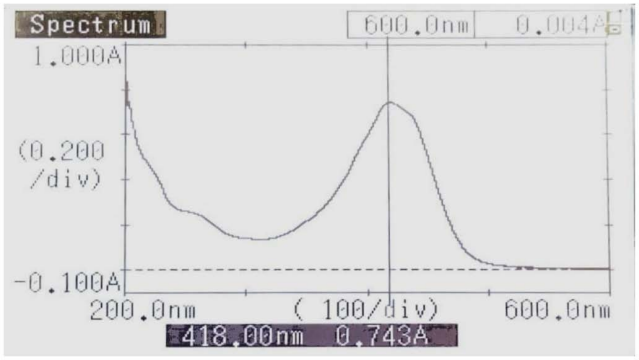

Figure 2: UV Absorption spectra of BBR at 418 nm

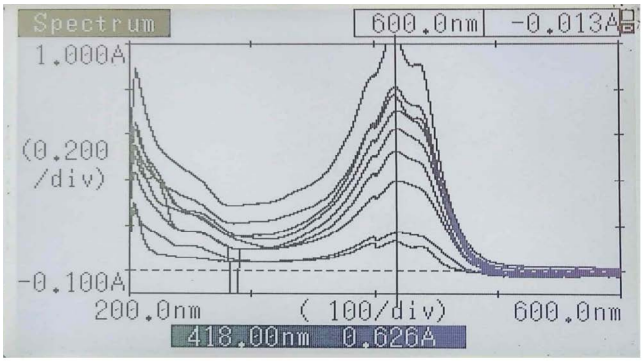

Figure 3: Overlay UV spectra of BBR in methanol showing maximum absorbance at 418 nm

Linearity

Dilutions spanning the concentration range of 1 to 15 µg/mL were analyzed and absorbance was noted in triplicate. The calibration curve was plotted and linear regression data demonstrated a strong linear relationship across the concentration range tested for BBR. The mean absorbance range (n = 3) was found to be 0.101 to 0.925, with RSD values below 2%, as shown in Table 2. The linear regression equation was determined as y = 0.0589x + 0.0591 with a coefficient of correlation value of R^2 = 0.9922 (Figure 4).

This linear relationship indicates that the method is capable of accurately quantifying BBR within the specified concentration range. The high R^2 value suggests that the model fits the data well, further validating the linearity of the method.

Precision

Following the procedure, three replicates of solutions containing BBR at concentrations 2, 10, and 15 µg/mL were prepared. The absorbance of each solution was measured at 418 nm to assess system precision. The %RSD was calculated, and it was found to be less than 2% as given in Table 3. To assess interday precision, three replicates of solutions containing BBR extract at concentrations of 2, 10, and 15 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 4) 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 BBR analysis.

Accuracy

The accuracy of the analytical method was evaluated through recovery experiments conducted at three distinct levels: 50, 100, and 150% of the expected sample concentrations. For each level, sample solutions were prepared and analyzed using the standardization approach. The amount of BBR in the sample solutions was consistent with the label claim of the formulation. The percent mean recovery of the sample was calculated, yielding a result of 99.64%. The percent recovery ranged between 99.64 and 100.39% (Table 5), indicating the excellent accuracy of the method. However, it is noteworthy that the %RSD was found to be greater than 2%, suggesting some variability in the recovery values.

This indicates that the method effectively quantifies BBR in the sample solutions, providing reliable results consistent with the expected concentrations. The observed accuracy validates the suitability of the UV method for precise and accurate determination of BBR content in pharmaceutical formulations.

Table 2: Linearity data of BBR

Concentration (µg/mL)	*Mean absorbance at 418 nm ± SD*	*%RSD*	*Regressed absorbance*
1	0.101 ± 0.001	0.990	0.118
2	0.15 ± 0.003	1.764	0.177
5	0.368 ± 0.001	0.272	0.353
7	0.502 ± 0.002	0.415	0.471
10	0.683 ± 0.002	0.293	0.647
12	0.782 ± 0.001	0.074	0.765
14	0.845 ± 0.002	0.237	0.882
15	0.925 ± 0.001	0.108	0.941

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

Table 3: Intraday precision data

Concentration (µg/mL)	*Abs 1*	*Abs 2*	*Abs 3*	*Mean Absorbance*	*S.D*	*%RSD*
2	0.152	0.149	0.151	0.151	0.002	1.014
10	0.684	0.685	0.683	0.684	0.001	0.146
15	0.926	0.928	0.925	0.926	0.002	0.165

Table 4: Interday precision data

Concentration (µg/mL)	*Abs 1*	*Abs 2*	*Abs 3*	*Mean Absorbance*	*S.D*	*%RSD*
2	0.15	0.153	0.152	0.152	0.002	1.007
	0.152	0.154	0.151	0.152	0.002	1.003
	0.155	0.151	0.154	0.153	0.002	1.358
10	0.686	0.684	0.68	0.683	0.003	0.447
	0.689	0.684	0.685	0.686	0.003	0.386
	0.683	0.686	0.681	0.683	0.003	0.368
15	0.924	0.92	0.926	0.923	0.003	0.331
	0.919	0.927	0.923	0.923	0.004	0.433
	0.927	0.923	0.925	0.925	0.002	0.216

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

Table 5: 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	10	15	14.95 ± 0.11	99.64	0.709
100	10	20	19.98 ± 0.12	99.88	0.580
150	10	25	25.20 ± 0.26	100.39	1.051

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

Table 6: Ruggedness study by two analysts

	Analyst 1	Analyst 2
	0.684	0.686
	0.687	0.684
	0.683	0.685
	0.685	0.683
	0.683	0.684
	0.686	0.687
Mean Absorbance	0.685	0.685
S.D	0.002	0.001
%RSD	0.239	0.215

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

Table 7: Robustness study of the proposed method

Condition	Parameter	Absorbance	Mean	SD	%RSD
Change in	417	0.745	0.751	0.01	1.544
Wavelength (nm)	418	0.743			
	419	0.764			
Change in	15	0.645	0.633	0.01	1.822
Temperature (°C)	25	0.632			
	35	0.622			

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 6), 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 BBR.

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 method's 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% as shown in Table 7. This indicates that the method is robust and capable of providing reliable results even under slightly altered experimental conditions.

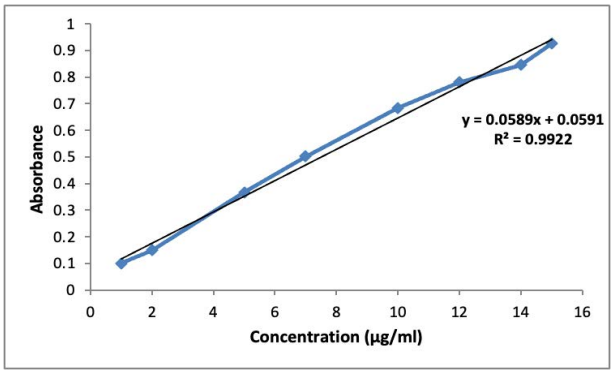

Figure 4: Calibration curve of BBR

Sensitivity

The limits of detection (LoD) and quantification (LoQ) for BBR were determined to be 1.565 and 4.742 µg/mL, respectively. These values represent the lowest concentration of BBR 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 BBR at trace levels in complex sample matrices. This demonstrates the method's suitability for applications requiring the detection of BBR at low concentrations, such as in pharmacokinetic studies or quality control analyses of herbal products.

CONCLUSION

In this study, an effective and simple UV spectrophotometric analytical technique was developed and validated for quantifying BBR. This method can be used to analyze BBR in different matrices, including plant extracts, pharmaceuticals, and biological samples, making it versatile. As compared to HPLC and electrochemical estimations UV method requires basic instrumentation and simple procedures. This method can also provide rapid results and requires minimal sample

preparation. Unlike chromatographic techniques, this UV method does not require elaborate treatment and complex procedures. It is less time-consuming and more economical, making it a highly efficient alternative for the quantitative analysis of BBR. The validation procedure conducted supports the suitability of this method for quantifying BBR in the formulation, making it a valuable tool for routine quality control in pharmaceutical settings.

ACKNOWLEDGEMENTS

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

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How to cite this article: Singh V, Chauhan I, Majhi S, Verma M, Yasir M. Development and Validation of UV-Spectrophotometric Method for Berberine Quantification. Journal of Applied Pharmaceutical Sciences and Research. 2024; 7(2):28-33 Doi : 10.31069/japsr.v7i2.05