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Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Berberinein Argemone Mexicanalinn.Extract

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ABSTRACT

A simple, rapid, precise and cost effective UV-visible spectrophotometric method for the estimation of Berberine in Argemone Mexicana Powder extracts. All the parameters of the analysis were chosen according to ICH Q2 (R1) guideline. Berberine solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of tangeretin were prepared. Calibration curve of concentration vs. absorbance was plotted. Various analytical method validation parameters were calculated. The maximum wavelength of Berberine was found to be 230 nm. The correlation coefficient at concentration range of 1-14 µg/ml was found to be 0.999. The validation study of the developed UV method was carried out by conducting linearity, accuracy, precision, robustness, ruggedness, limit of detection and limit of quantitation studies. Developed UV method was found to be precise for the intra and inter day studies and showed standard deviation in the range of 0.10 to 0.91 & 0.18 to 1.34 respectively. The total percent recovery of Berberine was found to be 99.71 to 101.14%. A simple, precise and cost-effective UV- visible spectrometry method was developed for the estimation of Berberine in standardized extract of Powder of Argemone mexicana. The said method was developed using co-solvent containing economical percentage of organic phase in aqueous media. The method was successfully used for the quantitative analysis of marketed formulation containing Argemone mexicanapowderand its extract. Due to sensitivity, proposed method can be used industrially for routine analysis of Berberine samples.

KEYWORDS: Argemone mexicana , Berberine, UV- Visible Spectrometric Method, Validation.

I. INTRODUCTION

Argemone mexicana(A.mexicana) Linn. (Papaveraceae) is a common plant found everywhere on roadsides and fields in India. The plant, Argemone mexicana Linn. belongs to the family Papaveraceae, is a widely distributed plant throughout the subtropical and tropical regions of the world. Argemone mexicana is considered as significant medicinal plant in India; the yellow juice, which exudes when the plant is injured, has long been used in India as traditional medicine for dropsy, jaundice, ophthalmia, scabies, and cutaneous affections [1-3]. Different parts of this plant are used in chronic skin diseases, and also as an emetic, expectorant, demulcent, and diuretic; the seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma, and other intestinal affections [4-5].

Berberine hydrochloride (BBR) (Fig. 1), a kind of isoquinoline alkaloid, is a commonly used drug extracted from a variety of herbs, including Coptidis rhizoma, Phellodendron Chinese schneid, and Phellodendron amurense [6-7]. It exhibited a variety of biological and pharmacological actions, such as antidiabetic activity [8-10], antitumor properties [10–12], bactericidal properties , as well as antiatherosclerotic activity , and anti-in amatory effects [12- 14]. It is soluble in an organic solvent like Ethanol and Methanol, Insoluble in water. Till date, there are reports of no economical UV-visible spectrophotometric method for estimation of BBR in extracts Argemone mexicana Linn. The said method was developed using a solvent containing an economical percentage of organic phase in aqueous media. Even, a precise economical UV-visible spectrophotometric method capable of estimating BBR in a variety of dosage forms like powder and solutions and the standardized extract is also unavailable. Therefore, considering the commercial importance and the needs of herbal

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industries, a simple yet precise and economical UV-visible spectrophotometric method capable of estimating BBR was developed and validated.

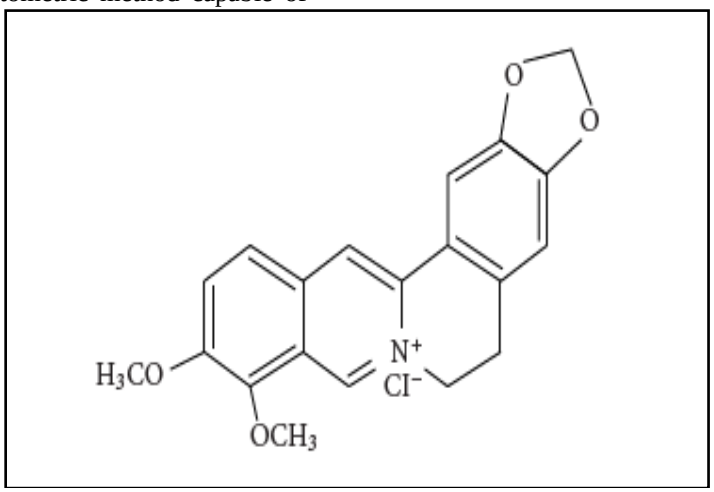

Fig. 1: Structure of Berberine hydrochloride

II. MATERIAL AND METHODS

BBR was purchased from TCI Chemicals (India) Pvt. Ltd, Chennai. Ethanol was purchase from Merck. All the chemicals of analytical grade were used for the proposed study.

Instruments Used

A double beam UV-visible spectrometer (UV-530, Jasco) connected to a computer loaded with spectra manager software was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Essae, Vibra HT) with internal calibration mode has been utilized for accurate weighing purposes.

Preparation of standard stock solution

Accurately weighed 1 mg of BBR was transferred into the calibrated volumetric flask and dissolved using a 1 ml co-solvent system consisting of ethanol and water (60:40 v/v) to achieve a stock solution of 1000 µg/ml (Stock-I). Stock- I solution was suitably diluted with a co-solvent system to achieve the solution of 100 µg/ml (Stock-II). Then Stock-II was further diluted to10 µg/ml (Stock-III)

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

The stock-III solution was scanned using the full scan method for the entire range of UV and visible i.e. 200 to 800 nm with the co-solvent system as a blank. After obtaining the spectrum, λmax was identified with the help of software the spectrum is shown in (fig.2). To achieve reproducible results, the above method was repeated five times.

Preparation of calibration curve

Calibration curve was prepared by diluting the stock-I solution to achieve the eighht different calibration standards representing 1, 2, 4, 6, 8, 10, 12 and 14 µg/ml strength. Absorbance of each calibration standard was measured at pre-identified λ_{max}; 230nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted. Above mentioned procedure was repeated three times so that reproducible results can be obtained.

Method Validation

The developed UV method for the estimation of BBR was validated as per the ICH guideline. Different parameters like linearity range, accuracy, precision, robustness, and ruggedness, the limit of detection (LOD), and the limit of quantitation (LOQ) were evaluated. [15-16]

Linearity and Range

The linearity of the proposed UV method was established using eight different calibration standards. Based on analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be an important factor for establishing the linearity of the proposed method. The interval between the upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of the analyte of interest. Three different

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solutions of BBR were prepared in triplicate at the level of 80%, 100%, and 120% of its predefined concentration. To the predefined concentrations, different amounts of BBR were added (standard addition method) and the accuracy was calculated based on percent recovery. For calculating the percent recovery following formula was used.

% RC= (SPS-S/SP) × 100

Where,

SPS = Amount found in the spiked sample

S = Amount found in the sample

SP = Amount added to the sample

% RC = Percent recovery

Precision

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of predefined samples. The study was performed at three concentration levels. The intra-day precision study was carried out by preparing nine different solutions of 1.5, 7 and 13 µg/ml strength of BBR (5 solutions of each concentration) and analyzing the same at morning, afternoon, and evening time of the same day. Deviation in the results was calculated in terms of % relative standard deviation (% RSD). Similarly, the inter-day precision study was carried out by analyzing the above-mentioned solutions on three consecutive days.

Robustness

The robustness of the developed UV method was established using different percentages of ethanol in the co-solvent system. Ethanol percentage in the co-solvent system was kept at 65 and 55% and BBR was dissolved in said co-solvent system separately. Triplicate samples were analyzed at 230 nm for absorbance. Levels of BBR in each sample were estimated using a respective calibration curve. The results were calculated in terms of % RSD.

Ruggedness

Ruggedness study of the method was carried out by analyzing triplicate samples of BBR solution (4 µg/ml) on two different Instruments (V-530, Jasco and BA-UV-2600, Bioage) and absorbance were noted in terms of % RSD.

Limit of Detection (LOD)

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

LOD=3.3×SD/S

Where, SD= Standard deviation of Y-intercepts

S= Slope

Limit of Quantitation (LOQ)

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

LOQ= 10×SD/S

Where, SD= Standard deviation of Y-intercepts

S= Slope

Estimation of BBR in A. mexicana extract

A. mexicana powder was dried at 50^0C using a Micro tray drier (S.B. Paschal and company, Mumbai, India) and powdered using a twin-blade mixer (Bajaj electrical ltd, Mumbai, India). To select uniform particle size, the powder was sifted in a sieve shaker (CIP Machineries, Ahmedabad, India) with sieves of different sizes (12, 24, 45, 85, and 120 mesh, Swastika electric and scientific works, Ambala, India) for 15 min. Powder passed through 120 mesh sieve was collected and used for further extraction. Soxhlet assisted extraction (SAE) technique was used for the extraction.10gm of powdered A. mexicana Powder was placed in a thimble (Borosil, Mumbai, India) which was inserted into a Soxhlet apparatus. The material was exhaustively extracted with ethanol. SAE was performed for 2 h. After a predefined extraction period, the solvent was distilled off under reduced pressure using a rotary vacuum evaporator (Heidolph Instruments GmbH & co. Germany) to obtain the dry extract. Accurately weighed 1 mg of dry extract of A. Mexicana Powder was transferred into the calibrated volumetric flask and dissolved using 1 ml of ethanol to achieve a stock solution of 1000 µg/ml (Stock-IV). Stock- IV solution was suitably diluted with a co-solvent system and analyzed for the BBR content using the proposed UV method.

III. RESULTS AND DISCUSSION

Determination of wavelength of maximum absorbance:

Identification of wavelength of maximum absorbance is a prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of the wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for BBR solution was carried out using full scan mode of UV-Visible spectrophotometer. The full scan was processed using UV software and the λmax was identified with the help of software. It was found to be 230 nm for BBR (Fig.2).

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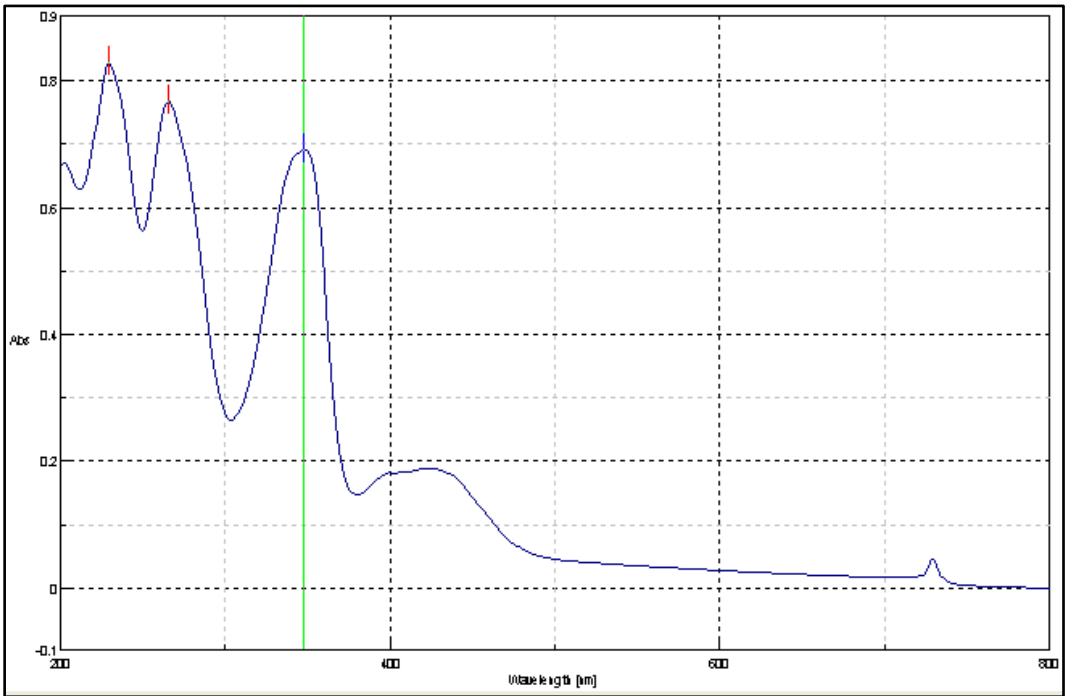

Fig. 2. UV-visible spectra of Berberine hydrochloride

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating the correlation between concentration and the response. As compared to the graphical method, above stated method is widely accepted and reproducible. Considering the utility of quantitative analysis of BBR, a calibration curve for BBR was developed using eight different calibration standards. The absorbance of different calibration standards at 230 nm was recorded using the fixed wavelength mode of UV-Visible spectrophotometer. The calibration curve was repeated three times and the mean values ± deviation was reported as shown in Table 1.

Table 1: Calibration standard data for BBR.

Concentration (µg/ml)	Absorbance
1	0.0764 ±0.048
2	0.1497 ±0.037
4	0.2918±0.029
6	0.4186 ±0.056
8	0.5309±0.042
10	0.6543±0.024
12	0.8071 ±0.061

Method validation

Linearity and Range

Linearity and range are the key parameters of the analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, an eight-point calibration curve of BBR covering a range of 01-12 µg/ml was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 1. The calibration curve when subjected to least square regression analysis yielded an equation; y = 0.064x + 0.020 with a correlation coefficient of 0.999 as shown in Fig. 3. From the linearity study, it was revealed that,

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developed UV method was linear in the pre-defined concentration range of calibration standards.

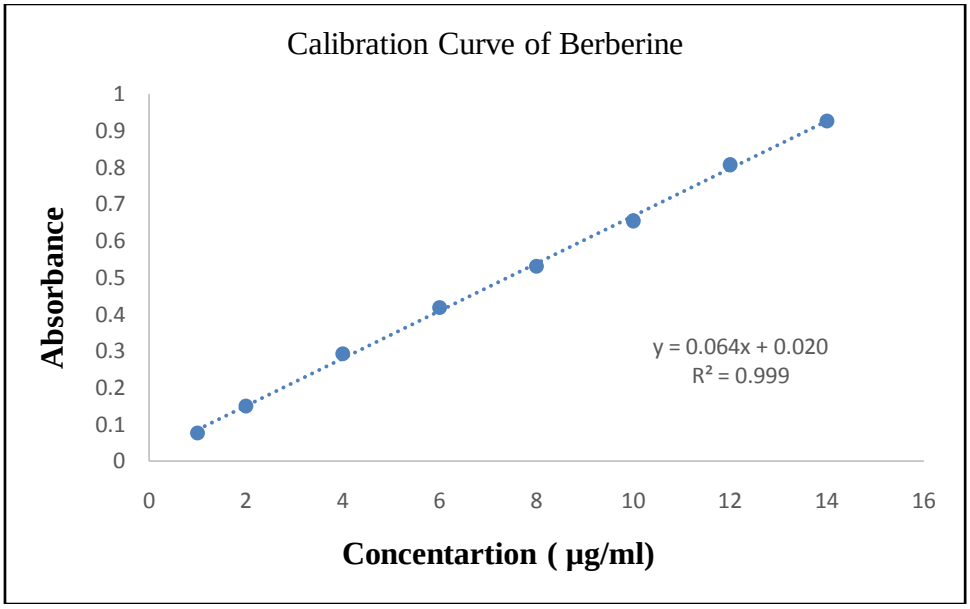

Fig. 3: Calibration curve for BBR.

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, the results obtained would be reliable. In the case of the UV method for BBR, accuracy was established using recovery studies. At 80 % standard addition, the mean recovery of BBR was found to be 100.37% whereas, at 100 and 120 % standard addition, it was found to be 101.14 and 99.71% respectively. % RSD was found to be less than 2 for the BBR recovery studies as shown in Table 2.

Table 2: Accuracy data of UV method for BBR

Concentration	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean Recovery	% RSD
80	1.5	1.2	101.62	100.37	1.3174
80	1.5	1.2	100.49		
80	1.5	1.2	99.00		
100	7	7	101.09	101.14	0.3343
100	7	7	101.50		
100	7	7	100.84		
120	13	15.6	99.62	99.71	0.6712
120	13	15.6	99.09		
120	13	15.6	100.42		

From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 102% and the % RSD was well below 2%.

Precision

Precision is a measure of the degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical

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method should generate reproducible outcomes. The precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of the developed UV method was established at 1.5, 7, and 13 µg/ml levels of BBR. The results in terms of mean absorbance values, percent assay, and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively.

Table 3: Intra-day precision data of UV method for BBR

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.51	100.66	0.4905	1.47	98.00	0.5528	1.49	99.33	0.2105
7	6.95	99.28	0.1914	6.98	99.71	0.1495	7.02	100.28	0.1778
13	13.17	101.30	0.1085	13.05	100.38	0.9186	13.13	101.00	0.2358

Table 4: Inter-day precision data of UV method for BBR

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.49	99.33	0.9480	1.47	98.00	0.1878	1.48	98.66	1.342
7	6.97	99.57	0.5173	6.93	99.00	0.9176	6.97	99.57	0.9325
13	13.11	100.84	0.8170	13.16	101.23	0.5115	13.02	100.15	0.6138

% RSD values of the intra-day precision study were found to be in between 0.10 and 0.91 whereas those of inter-day precision study was in between 0.18 and 1.34 Overall, % RSD values of less than 2 showed the precision of the developed UV method.

Robustness

The robustness of an analytical method is the ability of a method to resist the change in its performance despite the small, deliberate change in method parameters. It is an important parameter of the analytical method as a small, unintentional change in method parameters like solvent composition; pH, etc. may occur during routine use and may hamper the performance of the said method. It is expected that such change should not alter the performance of the analytical method. Therefore, a robust analytical method is preferred. The robustness of the proposed UV method was established by modifying the composition of the co-solvent system. Change in ethanol percentage (65 to 55 %) in the co-solvent system did not affect the method performance. % RSD values were found to be in between 0.12 and 1.11 as shown in Table 5. % RSD values below 2 showed that the proposed UV method is robust.

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Table 5: Robustness data of UV method for BBR

Robustness Study of BBR					
Concentration=7µg/ml					
Solvent Ratio (Ethanol: Water)	**I**	**II**	**III**	**Mean**	**%RSD**
(65:45)	0.4567	0.4664	0.4647	0.4626	1.1197
(60:40)	0.4392	0.4387	0.4398	0.439233	0.1253
(55:45)	0.4277	0.431	0.4217	0.450917	1.0456

Ruggedness

The ruggedness of the analytical method is the ability of a method to resist the change in its performance despite influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from the impact of environmental/external factors. To establish the ruggedness of the proposed UV method, the BBR solution was analyzed using two different UV-Visible spectrophotometers of two different labs. Sample analysis and data processing resulted in % RSD values between 0.29 and 1.01. Results revealed that the proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Table 6: Ruggedness data of UV method for BBR.

Ruggedness Study of BBR					
Concentration=7µg/ml					
Instrument	**I**	**II**	**III**	**Mean**	**%RSD**
Jasco	0.45097	0.4487	0.4487	0.449457	0.2915
Bioage	0.4113	0.4089	0.4178	0.412667	1.0158

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

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. LOD and LOQ of the proposed UV method were found to be 1.01 and 3.08 µg/ml respectively as shown in Table 7.

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

LOD	**1.0183**
LOQ	**3.0857**

Lower LOQ value indicated that the proposed method would be suitable for analyzing the samples containing even small quantities of BBR.

Estimation of BBR in A. Mexicana extract

The developed UV method was successfully applied for the estimation of BBR content in A. mexicana extract. By the proposed UV method, BBR content in Soxhlet extracts of A. Mexicana extract was found to be 0.020 ± 54.34 mg/g feed.

IV. CONCLUSION

A simple, accurate, and precise UV-Visible spectrophotometric method for the estimation of BBR in A. mexicana extracts was developed and validated. The proposed method was found to be robust and

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rugged in nature and was successfully used for the estimation of BBR present in A. mexicana extracts.

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