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Research

Development and Validation of Simple and Precise UV Spectroscopy Method for Quantifying Baicalein & Berberine in Bulk and Formulated Products

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Abstract

A simple, precise, and cost-effective UV-visible spectrophotometric method was developed and validated for the simultaneous estimation of baicalein and berberine in bulk and formulations, in accordance with ICH Q2 (R1) guidelines. Calibration standards of baicalein and berberine were prepared, and calibration curves of concentration versus absorbance were plotted. Various analytical method validation parameters such as linearity, accuracy, precision, robustness, ruggedness, limit of detection, and limit of quantitation were calculated. The maximum wavelengths for baicalein and berberine were found to be 274 nm and 344 nm, respectively. The correlation coefficients over the concentration ranges of 0.5-8 µg/ml and 5-30 µg/ml were 0.998 and 0.999 for baicalein and berberine, respectively. The developed UV method demonstrated high precision, accuracy, and sensitivity, with % RSD less than 2. This validated UV-visible method can be efficiently used for the individual and simultaneous estimation of baicalein and berberine in bulk and formulations.

Keywords: UV Spectra, Baicalein, Berberine, LOQ, LOD, Herbal formulations

INTRODUCTION

The UV spectroscopic method provides a reliable means for quantitative analysis in pharmaceutical and chemical research. It involves measuring the absorption of ultraviolet light by molecules, allowing for the determination of concentrations in solutions based on established calibration curves. This method is widely employed due to its sensitivity, simplicity, and ability to analyze a broad range of substances with distinct UV absorption spectra, making it invaluable in various fields including pharmaceutical formulation and quality control. Baicalein, also known as Shyonaka and Sonapath, is a flavone derived from the leaves of Oroxylum indicum, a member of the Bignoniaceae family. Found predominantly in the root bark, seeds, and leaves of this

plant, baicalein has garnered significant attention among natural product researchers due to its diverse pharmacological properties. It is chemically described by the formula $C_{15}H_{10}O_{5}$[1] and is recognized for its therapeutic potential in treating conditions such as pulmonary artery diseases, Alzheimer's disease, and Parkinson's disease[2]. Baicalein exhibits a range of pharmacological effects including antioxidative, anti-inflammatory, anti-viral, anti-bacterial, anticancer, and anti-allergic properties, among others. Soluble in organic solvents like ethanol, methanol, and water, its abundance in various foods and plants underscores its value as a dietary supplement, driving the commercial interest in standardized extracts of Oroxylum indicum [3]. Berberine, scientifically known as a quaternary ammonium salt derivative of isoquinoline alkaloid, is originally isolated from the roots, rhizomes, and stem bark of various plants such as Berberis species, Phellodendron amurense, and Coptis chinensis. Belonging to the family Berberidaceae, berberine has garnered significant attention in natural product research due to its diverse pharmacological effects. Berberine is chemically represented by the formula $C_{20}H_{18}NO_4^+$ [4]. This compound has shown promising therapeutic potential in the treatment of conditions such as diabetes, cardiovascular diseases, and gastrointestinal infections. Its pharmacological actions include anti-diabetic, lipid-lowering, anti-inflammatory, antimicrobial, and antioxidant properties, among others [5]. Berberine exhibits solubility in water and various organic solvents, facilitating its formulation into different pharmaceutical and dietary supplement products. Its presence in multiple plant sources underscores its importance as a key bioactive compound in traditional medicine and modern healthcare [6]. An accurate UV-visible spectrophotometric method has been developed and validated for the precise estimation of Baicalein and berberine in various dosage forms such as powders and solutions. This method addresses the commercial significance and the requirements of herbal industries, offering a straightforward, cost-effective solution for their quantitative analysis needs.

MATERIALS AND METHOD

Materials

Baicalein and Berberine were sourced from TCI Ltd, India. Marketed Berberine & Baicalein capsule were purchased from Livestamin Healthcare LLP and Swanson Health Products respectively. All chemicals used in this study were of analytical grade.

Instruments used

A double beam UV-visible spectrophotometer (Shimadzu 1900i) with spectra manager software was used for the analysis. Quartz cells having 1 cm path length with 3 cm length were used for spectral measurement. Sonicator (Verilux ® 0.8L Mini Ultrasonic) Weighing balance (Wensar PGB 600) with internal calibration mode was used for the accurate weighing purpose.

Standard Curve of Baicalein & Berberine Using UV Spectra

Preparation of standard solution and Calibration curve of baicalin

An accurately weighed quantity (1mg) of baicalein was dissolved in distilled water and made up to 200ml with distilled water in a volumetric flask and designed as Stock solution-1 Into series of the volumetric flasks (100ml), aliquots of the sample (25, 50, 75 & 100 ml) were taken from stock solution-1 and made up to 100ml with distilled water to obtain 1.25, 2.5, 3.75, 5 µg/ml concentrations respectively. The aliquot was scanned between 200-400nm. The λ max obtained for baicalein is 274nm.

The absorbance values at the λmax 274 nm obtained in each case was plotted against concentration to obtain a calibration curve.

Preparation of standard solution and Calibration curve of berberine

An accurately weighed quantity (2mg) of berberine was dissolved in distilled water and made up to 50ml with distilled water in a volumetric flask and designed as Stock solution-1 Into series of the volumetric flasks (10ml), aliquots of the sample (1.25, 2.5, 5, 7.5 &10 ml) were taken from stock solution-1 and made up to 10ml with distilled water to obtain 5, 10, 20, 30 & 40µg/ml concentrations respectively. The aliquot was scanned between 200-400nm. The λ max was observed at 344nm for berberine.

The absorbance values at the λmax 344 nm obtained in each case was plotted against concentration to obtain a calibration curve. The linear correlation between these concentrations(x- axis) and absorbance (y-axis) were graphically presented and slope (b), intercept (a), and correlation coefficient (r^2) were calculated for the linear equation (y=bx+a)

Validation of the developed method [8-9]

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

Accuracy

To the preanalysed sample solutions, a known amount of standard stock solution of baicalein was added at different levels, i.e. 100%, and 120%. The solutions were reanalyzed by the proposed method. The same procedure was adapted to berberine stock solution.

Precision

Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 2, 4 and 6 µg/ml of baicalein solutions for three times in the same day. Interday precision was determined by analyzing the 2, 4 and 6 µg/ml of baicalein solution daily for 3 days over the period of week. The same procedure was adapted to berberine with 5, 10, and 30µg/ml concentrations.

Sensitivity

The sensitivity of measurements of baicalein 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 equation

Quantitation Limit (LOQ) may be expressed as:
$LOQ = 10\sigma/S$
Where
σ = Relative standard deviation of the response.
S = the slope of the calibration curve (of the analyte).
SD of intercept= SE of intercept X $\sqrt{N}$
LOD= 3.3X (SD of intercept/ slope)

Repeatability

Repeatability was determined by analyzing 6 µg/ml and 10 µg/ml concentrations of baicalein and berberine solution respectively for six times.

Ruggedness

of the proposed method is determined for 6µg/ml concentration of baicalein and 10 µg/ml concentration berberine by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions.

Determination of baicalein and berberine in bulk

Accurately weighed 1 mg of baicalein was transferred into a 100 ml volumetric flask containing 10 ml distilled water, and the volume was made up to the mark using the same. Appropriate volume 4 ml of this solution was transferred to a 10 ml volumetric flask and the volume were adjusted to the mark using distilled water. Absorbance was read by 274nm. In the case of berberine 1mg accurately weighed and transferred into a 50 ml volumetric flask containing 50 ml distilled water, and the volume was made up to the mark using the same. The absorbance values are obtained at 344nm. The concentrations of the drug were calculated from linear regression equations.

Application of the proposed method for pharmaceutical formulation

For analysis of commercial formulation 1 mg equivalent weight of baicalein was taken in a 250 ml volumetric flask and the volume was made up to the mark with distilled water to give 4 µg/ml concentration and 1 mg equivalent weight of berberine was taken in a 50 ml volumetric flask and the volume was made up to the mark with distilled water to give 20 µg/ml concentration. The spectrum was recorded at 274 nm for former and 344nm for later. The concentrations of the drug were calculated from the linear regression equation.

RESULTS AND DISCUSSION

Determination of Wavelength of Maximum Absorbance

Identifying the wavelength of maximum absorbance is essential for quantitative UV analysis. A solution with an absorbance value less than 1 is typically considered suitable for this determination. Considering these criteria, the maximum wavelength for a baicalein solution (2 µg/ml) and berberine (5µg/ml) were identified using the full scan mode of a UV-Visible spectrophotometer (Figure 1&2). The full scan was processed with Shimadzu 1900i software, which identified the λmax. The λmax for baicalein and berberine were found to be 274 nm and 344nm respectively.

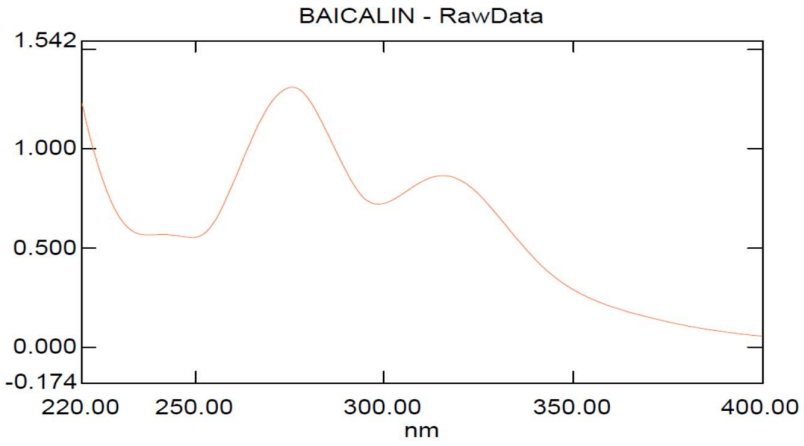

Fig 1: UV-visible spectra of Baicalein

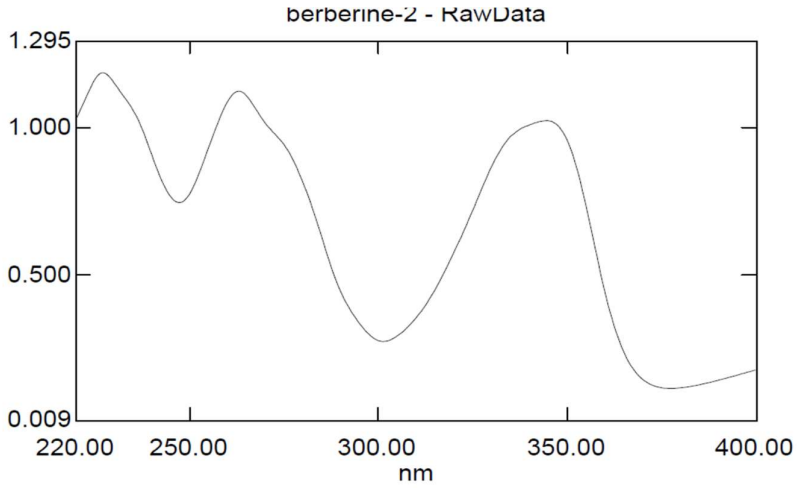

Fig 2: UV-visible spectra of Berberine

Preparation of Calibration Curve

Quantification of unknown samples by UV-Visible spectrophotometry or any other instrumental method requires a reproducible calibration curve and a mathematical equation correlating concentration with response. This method is preferred over graphical methods for its reproducibility. For quantitative analysis of Baicalein and Berberine, a calibration curve were developed using four different calibration standards. The absorbance of these standards at 274 nm [9] and 344nm [10] respectively were recorded using the fixed wavelength mode. The calibration curve was generated five times, and the mean values ± standard deviation was calculated.

Table 1: Calibration standard data for Baicalein & Berberine

Slno	Baicalein		Berberine	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	1.25	0.189±0.03	5	0.121±0.02
2	2.5	0.381±0.05	10	0.246±0.01
3	5	0.732±0.07	20	0.482±0.04
4	7.5	0.992±0.01	30	0.767±0.12
5	10	1.320±0.02	40	0.996±0.10

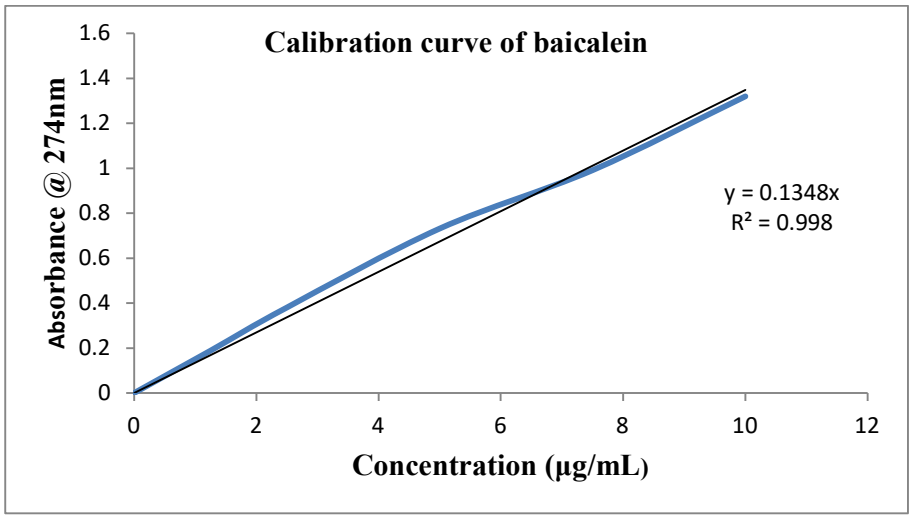

Fig 3: Calibration curve of baicalein

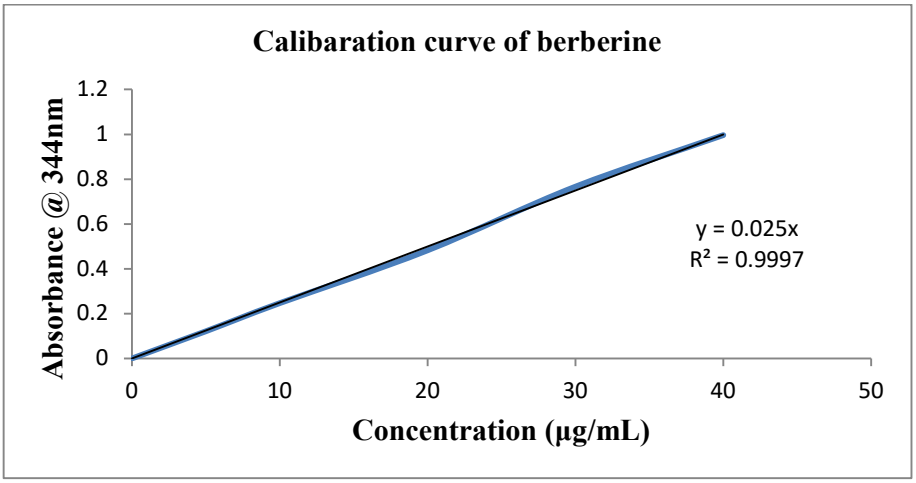

Fig 4: Calibration curve of berberine

Validation

The linear regression data for the calibration curves showed good linear relationship over the concentration range 1–7.5 and 2-40µg/ml for baicalein and berberine respectively [Figure3& 4]. Linear regression equation was found to be y = 0.1348x (*r*2 = 0.996) and y = 0.025x (*r*2 = 0.999). The result is presented in Table 2.

Table 2: Results of linearity, LOD and LOQ of baicalein and berberine

Parameter	Observation	
	Baicalein	**Berberine**
λ_{max} (nm)	274nm	344nm
Beer's Linearity (µg/ml)	1 – 7.5	2-40
Regression equation (Y= mx + c)	y = 0.1348x	y = 0.025x
Slope(m)	0.130 ± 0.00388	0.0251 ± 0.000359
Correlation coefficient (r^2)	0.996	0.999
SE intercept	0.0258	0.01191
LOD	0.28253	0.703095
LOQ	0.856152	2.13059

Accuracy

The solutions were reanalyzed by the proposed method; results of recovery studies are reported in Table 3 which showed that the % amount found was between 100.5and 101% with % RSD > 2and the % amount found was between 100.5and 102.7 % with % RSD > 2 for baicalein and berberine respectively.

Table 3: Results of Recovery studies

Pre-analyzed sample	Amount of drug added (µg/ml)n=3	Amount recovered (µg/ml) n=3	% Recovery	% RSD
Baicalein (%)				
100	0	2.01±0.02	100.5	1.34
150	.2	2.02±0.03	101	1.49
Berberine (%)				
100	0	9.17±0.065	100.5±0.95	0.949
120	4.5	4.67±0.13	102.7±1.30	1.28

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 that indicate this method precise for the determination of both the drugs [Table 4].

Table 4: Results of precision studies

Component	Concentration (µg/ml)	Interday precision n=3		Interday precision n=3	
		Conc. found	%RSD	Conc. found	%RSD
Baicalein	2	2.02±0.03	1.78	2.03±0.04	1.97
	4	4.01±0.30	0.76	4.06±0.07	1.73
	6	6.08±0.12	2.0	6.01±0.12	2.0
Berberine	5	5.02±0.10	2.0	5.03±0.06	1.35
	10	10.6±1.21	1.21	10.13±0.10	1.01
	30	30.37±0.56	1.86	30.4±0.61	2.0

Repeatability

Repeatability was assessed by analyzing 6 µg/ml and 10 µg/ml concentrations of baicalein and berberine solutions six times. The percentage amounts found were between% and% with% RSD < 2 and% and% with% RSD < 2 respectively [Table 5].

Table 5: Results of repeatability studies

Component	Amount taken (µg/ml) (n-=6)	Amount found (%)	% RSD
Baicalein	6	6.03±0.05	0.95
Berberine	10	10.08±0.138	1.37

* Average of six estimations

Ruggedness

The absorbance was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 6. The result showed that the % RSD was less than 2%.

Table 6: Results of ruggedness studies

Component	Amount taken (µg/ml) (n-=3)	Amount found (%)	
		Analyst-1±SD	Analyst-2±SD
Baicalein	6	6.07±0.04	6.04±0.06
Berberine	10	10.1±0.138	9.96±0.29

Determination of baicalein and berberine in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 100& 103% and 99.4& 102.2% [Table 7].

Table 7: Estimation of baicalein & berberine in bulk

Concentration (µg/ml)	Amount found (µg)	Amount found (%)
Baicalein		
4	4.12	103.0
4	4.00	100.0
4	4.02	100.5
Mean	**4.04**	101. 1
%RSD	1.59	1.59
Berberine		
20	20.44	102.2
20	20.12	100.6
20	19.88	99.4
Mean	**20.14**	**100.73**
%RSD	1.44	1.39

Application of the proposed method for pharmaceutical formulation

The spectrum was recorded at 274 nm and 344nm for baicalein & berberine. The concentrations of the drug were calculated from the linear regression equation. The % amount found was between 99.7% and 103% and between 99.5% and 103.1% [Table 8].

Table 8: Estimation of baicalein & berberine in formulations

Concentration (µg/ml)	Amount found (µg)	Amount found (%)
Baicalein		
4	4.12	103.0
4	4.10	102.5
4	3.99	99.7
Mean	4.07	**101.7**
%RSD	1.72	**1.75**
Berberine		
20	20.12	100.6
20	19.90	99.50
20	20.63	103.1
Mean	20.21	**100.06**
%RSD	1.85	**1.83**

CONCLUSION

In conclusion, Baicalein and Berberine, known for their limited solubility in water (0.18 mg/g and 2 µg/ml, respectively), were successfully analyzed using a UV methodology developed in this study. This method allows simultaneous detection of both drugs without the need for organic solvents, employing a straightforward analytical approach. The findings demonstrate that the UV-spectrophotometric technique developed is simple, accurate, precise, reproducible, and sensitive. This method proves effective for quantifying Baicalein and Berberine, either together or individually, across various formulations, including herbal extracts.

Declaration of Interest

The authors declare no conflicts of interest related to this work. The responsibility for the content and writing of this paper lies solely with the authors.

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