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Development and validation of UV-spectrophotometric method for estimation of Baicalein in *Oroxylum indicum* leaf extract

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Abstract

A simple, precise and cost effective UV- visible spectrophotometric method for the estimation of Baicalein in *Oroxylum indicum* leaf extracts was developed and validated according to the ICH Q2 (R1) guideline. Baicalein (5, 6, 7- trihydroxyflavone) is a flavone isolated from the leaves of *Oroxylum indicum* plant. Spiked Baicalein solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of Baicalein were prepared. Calibration curve of concentration vs. absorbance was plotted. Various analytical method validation parameters viz. linearity, accuracy, precision, robustness, ruggedness, limit of detection and limit of quantitation were calculated. The maximum wavelength of Baicalein was found to be 274 nm. The correlation coefficient over the concentration range of 0.5-8 µg/ml was found to be 0.999. Developed UV method was found to be precise for the intra-day and inter-day study and shows percent relative standard deviation in the range of 0.498 & 1.809 to 0.809 to 1.87 respectively. The total percent recovery of Baicalein was found to be 98.96 to 99.27%. A simple, precise and cost effective UV- visible spectrophotometric method for the estimation of Baicalein in standardized extract of leaves of *Oroxylum indicum* was developed. The said method was developed using solvent containing economical percentage of organic phase in aqueous media. The present validated UV- visible method can be efficiently used for the estimation of Baicalein in extracts of leaves *Oroxylum indicum*.

Keywords: UV- visible spectrophotometry, Baicalein, *Oroxylum indicum*, validation

Introduction

Baicalein (5, 6, 7 –trihydroxyflavone) is a flavone, originally isolated from the leaves of *Oroxylum indicum*. Baicalein commonly known as Shyonaka and Sonapath, belongs to family Bignoniaceae. Baicalein is present in root bark, seeds, and leaves of *Oroxylum indicum*. Leaves are consisting of variety of chemicals with wide range of activities among all the chemicals in leaves; Baicalein has gained the attention of researchers working in natural products. It is a derivative of flavone glycoside and its chemical formula is C₁₅H₁₀O₅ [1, 2]. It is generally use for the treatments of pulmonary artery, Alzheimer disease, and Parkinson disease. It has several pharmacological effects viz. reducing oxidative stress, anti-inflammatory, inhibiting excitotoxicity and anti-oxidant, anti-viral, anti-bacterial, anticancer, and anti-allergic therapies, anti-apoptosis effects [3, 4]. It is soluble in organic solvent like ethanol, methanol and water. Abundant presence of Baicalein in several foods and plants including imparts prominent dietary supplement activity to extracts therefore standardized *Oroxylum indicum* extracts are gaining commercial importance [5, 6]. Till today, there is no single UV-visible spectrophotometric method available for estimation of Baicalein in extracts of *Oroxylum indicum* leaves. Even, a precise UV- visible spectrophotometric method capable of estimating Baicalein in variety of dosage forms like powder and solutions is available in market. Therefore, considering the commercial importance and the needs of herbal industries, a simple yet precise and economical UV-visible spectrophotometric method capable of estimating Baicalein was developed and validated.

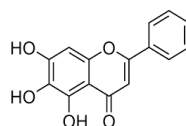


Fig 1: Chemical Structure of Baicalein

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Materials and Method

Materials

The standard of Baicalein (purity 98% by HPLC) was purchased from Sigma-Aldrich (St-Louis, MD, USA). Dimethylformamide and Methanol was purchase from Molychem & Merck. All the chemicals of analytical grade were use for the proposed study.

Instruments used

A double beam UV-visible spectrophotometer (UV-530, Jasco) with spectra manager software were used for the analysis. Quartz cells having 1 cm path length with 3 cm length were used for spectral measurement. Weighing balance (Essae, Vibra HT) with internal calibration mode was used for the accurate weighing purpose.

Preparation of standard stock solution

Accurately weighed 1 mg of Baicalein was transferred in to the calibrated volumetric flask and dissolved using 1 ml of Dimethylformamide to achieve a stock solution of 1000 µg/ml (Stock-I). Stock- I solution was suitably diluted with co-solvent system methanol and water (25:75 v/v) to achieve solution of 100 µg/ml (Stock-II).

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

Stock-II solution was scanned using full scan mode for the entire range of UV and visible i.e. 400 to 200 nm with co-solvent system as a blank. After obtaining the spectrum, λ_{max} was identified with the help of software. In order to achieve reproducible results, above method was repeated five times.

Preparation of calibration curve

Calibration curve was prepared by diluting the stock-II solution to achieve the seven different calibration standards representing 0.5, 1, 2, 3, 4, 6, 8 µg/ml strength. Absorbance of each calibration standard was measured at pre-identified λ_{max} 274 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted. Above mentioned procedure was repeated pentaplicate so that reproducible results can be obtained.

Method Validation

Developed UV method for the estimation of Baicalein was validated as per the ICH guideline. Different parameters like linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated [9, 10].

Linearity and Range

Linearity of the proposed UV method was established using seven 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 important factor for establishing linearity of the proposed method. The interval between lower and upper 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 analyte of interest. Three different solutions of Baicalein were prepared in triplicate at level of 80%, 100% and 120% of its predefined

concentration. To the predefined concentrations, different amounts of Baicalein were added (standard addition method) and the accuracy was calculated based on percent recovery. For calculating the percent, recovery following formula was used.

$$\% RC = (SPS - S / SP) \times 100$$

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. Intra-day precision study was carried out by preparing nine different Baicalein solutions of 1, 3 and 7 µg/ml strength (3 solutions of each concentration) and analyzing the same at morning, afternoon and evening time of same day. Deviation in the results was calculated in terms of% relative standard deviation (% RSD). Similarly, inter-day precision study was carried out by analyzing the above mentioned solutions at three consecutive days.

Robustness

Robustness of the developed UV method was established using different percentage of methanol in co-solvent system. Methanol percentage in co-solvent system was intentionally adjusted to 23 and 28% and middle level quality control sample (3 µg/ml) of Baicalein was prepared using above mentioned co-solvent system separately. Samples (n=5) were analyzed at 274nm for Baicalein content. The results were calculated in terms of% RSD.

Ruggedness

Ruggedness study of the method was carried out by analyzing triplicate samples of Baicalein solution (3µg/ml) using two different instruments (V-530, Jasco and BA-UV-2600, Bioage). Results were expressed in terms of% RSD

Limit of Detection (LOD)

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

$$LOD = 3.3 \times SD / S$$

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

$$LOQ = 10 \times SD / S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Estimation of Baicalein in *Oroxylum indicum* extracts

Oroxylum indicum leaves were dried at 50°C using a Microtray drier (S.B. Panchal and company, Mumbai, India) and powdered using twin blade mixer (Bajaj electrical ltd., Mumbai, India). To select uniform particle size, 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 a period of 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 of *Oroxylum indicum* leaves. 60 gm of powdered *Oroxylum indicum* leaves was placed in a thimble (Borosil, Mumbai, India) which was inserted into a Soxhlet apparatus and extracted with 180 ml ethanol as an extraction solvent. SAE was performed for 5 hrs. After predefined extraction period, solvent was distilled off under reduced pressure using rotary vacuum evaporator (Heidolph instruments GmbH & co. Germany) to obtain the dry extract. Accurately weighed 1 mg of dry extract of *Oroxylum indicum* was transferred in to the calibrated volumetric flask and dissolved using 1 ml of Dimethylformamide (DMF) to achieve a stock solution of 1000 µg/ml (Stock-I). Stock- II solution was suitably diluted with co-solvent system and analyzed for the Baicalein content using proposed UV method.

Results and Discussion

Determination of wavelength of maximum absorbance

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for Baicalein solution (5 µg/ml) was carried out using full scan mode of UV-Visible spectrophotometer (figure 2). Full scan was processed using Jasco UV software and the λ_{max} was identified with the help of software. The λ_{max} was found to be 274 nm for Budesonide.

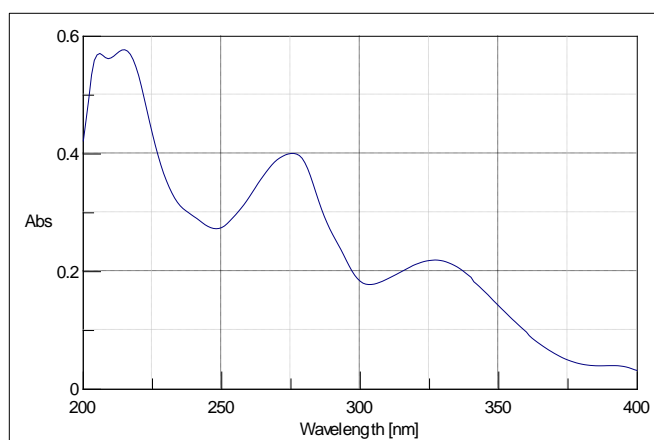


Fig 2: UV-visible spectra of Baicalein

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 a mathematical equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Baicalein, calibration curve for Baicalein was developed using seven different calibration standards. The absorbance of different calibration standards at 274 nm was recorded using fixed wavelength mode. Calibration curve was repeated five times and the mean values \pm standard deviation

was reported as shown in Table 1.

Table 1: Calibration standard data for Baicalein

S. No.	Concentration (µg/ml)	Absorbance
1	0.5	0.0495 \pm 0.0022
2	1	0.1156 \pm 0.0042
3	2	0.2456 \pm 0.0041
4	3	0.3529 \pm 0.0055
5	4	0.4765 \pm 0.0062
6	6	0.7328 \pm 0.0068
7	8	0.9533 \pm 0.0071

Method Validation

Linearity and Range

Linearity and range are the key parameters of 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, seven-point calibration curve of Baicalein covering a range of 0.5-8µg/ml was plotted. Details of concentrations and the respective mean absorbance values are shown in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; $y = 0.1209x + 0.005$ with correlation coefficient 0.999 as shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear in the pre-defined concentration range of calibration standards.

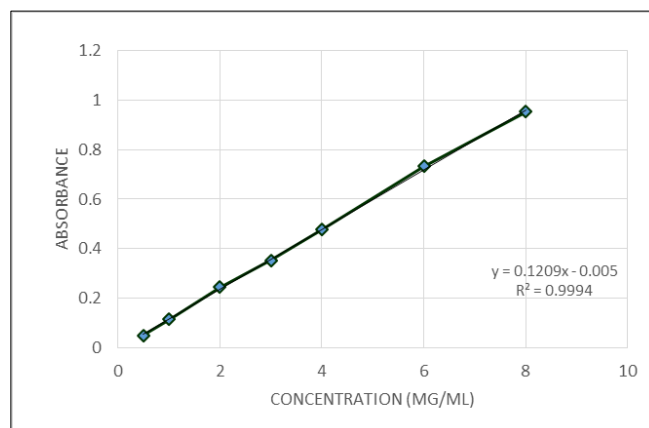


Fig 3: Calibration curve for Baicalein

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, results obtained would be reliable. In case of UV method for Baicalein, accuracy was established using recovery studies. At 80% standard addition, mean recovery of Baicalein was found to be 99.27% whereas at 100 and 120% standard addition, it was found to be 98.96 and 99.21% respectively. % RSD was found to be less than 2 for the Baicalein recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 97 to 100% and the % RSD was well below 2%.

Table 2: Accuracy data of UV method for Baicalein

Sr No.	Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean% Recovery	% RSD
1	80	1	0.8	98.81	99.27	1.70
2	80	1	0.8	100		
3	80	1	0.8	98.1		
4	100	3	3	100	98.96	1.97
5	100	3	3	98.6		
6	100	3	3	98.5		
7	120	7	7.2	100	99.21	1.63
8	120	7	7.2	97.4		
9	120	7	7.2	99.4		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 1, 3 and 7 µg/ml levels of

Baicalein. 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. % RSD values of intra-day precision study were found to be in between 0.87 and 1.95 whereas those of inter-day precision study were in between 0.49 and 1.74. Overall, % RSD values of less than 2 demonstrated the precision of developed UV method.

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

S. NO	Concentration Range (µg/ml)	Morning			Afternoon			Evening		
		Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	1	0.1127	97.56	1.792	0.1138	98.44	1.615	0.1139	98.56	1.8099
2	3	0.3378	97.47	1.768	0.3417	98.53	1.669	0.3370	97.17	1.4693
3	7	0.8088	99.96	0.9805	0.8096	100	0.498	0.8103	100	1.2677

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

S. No.	Concentration Range (µg/ml)	Day 1			Day 2			Day 3		
		Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	1	0.1138	98.42	1.872	0.1154	99.82	0.8921	0.1139	98.56	1.8099
2	3	0.3413	98.42	1.624	0.3381	97.50	1.0388	0.3370	97.46	1.469
3	7	0.8203	100	1.580	0.8097	100	0.5673	0.8103	100	1.2677

Robustness

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition; pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the analytical method. Therefore, robust analytical method is preferred. Robustness of proposed UV method was established by modifying the composition of co-solvent system. Change in methanol percentage (23 to 28%) in co-solvent system did not affect the method performance. % RSD values were found to be in between 1.96 and 1.24 as shown in Table 5. % RSD values below 2 showed that proposed UV method is robust in nature.

Table 5: Robustness data of UV method for Baicalein

S. No.	Concentration (µg/ml)	% Methanol	Mean Absorbance	% RSD
1	3	23	0.3082	1.96
2	3	28	0.3632	1.24

Ruggedness

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from impact of environmental/external factors. In order to establish the

ruggedness of proposed UV method, Baicalein solution was analyzed at three different temperature conditions. Sample analysis and data processing resulted into % RSD values between 0.63 and 1.48. Results revealed that 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 Baicalein

S. No.	Concentration (µg/mL)	Instrument	Mean Absorbance	% RSD
1	3	Jasco	0.3618	1.15
2	3	Bio-age	0.4238	1.74

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

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.22 and 0.67 µg/ml respectively as shown in Table 7. Lower LOQ value indicated that proposed method would be suitable for analyzing the samples containing even small quantities of Baicalein.

Table 7: LOD & LOQ data for UV method for Baicalein

1	LOD	0.22 µg/ml
2	LOQ	0.67 µg/ml

Estimation of Baicalein in *Oroxylum indicum* extracts

Developed UV method was successfully applied for

estimation of Baicalein content in *Oroxylum indicum* leaves extracts. By proposed UV method, Baicalein content in Soxhlet extracts of *Oroxylum indicum* was found to be 1.57 ± 0.0058 mg/g feed.

Conclusion

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of Baicalein was developed and validated. The Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of Biacalein.

References

1. Lawania R, Mishra A, Gupta R. *Oroxylum indicum*: A Review. Pharmacognosy Journal. 2010; 2(9):304-310.
2. Preeti A, Sharma S. A review on *Oroxylum indicum* (L.) Vent: an important medicinal tree. International Journal of Research in Biological Sciences. 2016; 6(1):7-12.
3. Satya Eswari J, Dhagat S, Naik S, Dibya S. *Oroxylum indicum* leaf extracts for screening of antimicrobial properties and phytochemicals. Pharm. Bioprocess. 2018; 6(1):7-14.
4. Zaveri M, Gohil P, Jain P. Immunostimulant Activity of n-Butanol Fraction of Root Bark of *Oroxylum indicum*, vent. Journal of Immuno toxicology. 2006; 3:83-99.
5. Upaganlawar A, Tenpe CR, Yeole PG. Anti-inflammatory activity of aqueous extract of *Oroxylum indicum* vent. Leaves extract- preliminary study. Pharmacologyonline. 2009; 1:22-26.
6. Zaveria M, Khandhar A, Jain S. Quantification of Baicalein, Chrysin, Biochanin-A and Ellagic Acid in Root Bark of *Oroxylum indicum* by RP-HPLC with UV Detection. Eurasian Journal of Analytical Chemistry. 2008; 3(2):25-257.
7. Maddi Sesharao, Vallabaneni Madhavarao. A new validated simultaneous reversed-phase high-performance liquid chromatography assay method for estimation of two flavones (baicalein and Schrysin) in API drugs. Asian Journal of Pharmaceutical and Clinical Research. 2018; 11(1):351-356.
8. Bhusari SS, Rindhe M, Chaudhary A. Development and validation of UV-Spectrophotometric method for estimation of Picroside-ii in Picrorhiza Kurroa rhizome extracts. International Journal of Pharmacy and Biological Sciences. 2018; 8(4):177-183.
9. Note for guidance on validation of analytical procedures: text and methodology. European Medicines Agency, 1995, 1-15.
10. ICH Guidance on Analytical Method Validation, In: Proceedings of the international Conference of Harmonization, Geneva, 1996.