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Research Article

Validated UV Visible Spectrophotometric Method for The Estimation of Picroside-II in Its In-House Proliposomes and The Commercial Formulations

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ABSTRACT

A simple, accurate, precise and cost-effective UV spectrophotometric method for the determination of Picroside-II (PK-II) in proliposomal and herbal formulations was developed and validated. PK-II is the iridoid glycoside mainly found in the rhizomes of Picrorhiza kurroa. The wavelength of maximum absorbance of PK-II was observed to be 218 nm in deionized water. Method validation included assessment of linearity, accuracy, precision, limit of detection, limit of quantification, ruggedness, and robustness. The method demonstrated excellent linearity (1-30 µg/mL) with a correlation coefficient (R2 = 0.999). Accuracy was confirmed by low %Difference, while inter-day and intra-day precision showed low %RSD values. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.6470 µg/mL and 1.9606 µg/mL, respectively. The validated method proved to be both robust and rugged, making it suitable for routine analysis of PK-II in proliposomal and herbal formulations.

INTRODUCTION

Picrorhiza kurroa, commonly known as Kutki, is a perennial herb primarily found in India[1]. Its elongated rhizomes contain numerous phytoconstituents, including Picroside-I (PK-I), Picroside-II (PK-II)[2]. Among these, Picroside-II (PK-II) is one of the most abundant. PK-II is a pale yellow to white crystalline compound belonging to the glycoside category, with the chemical formula $C_{23}H_{28}O_{15}$. It has a log P value of -0.09675 and a pKa value of 7.80, indicating its hydrophilic nature[3].

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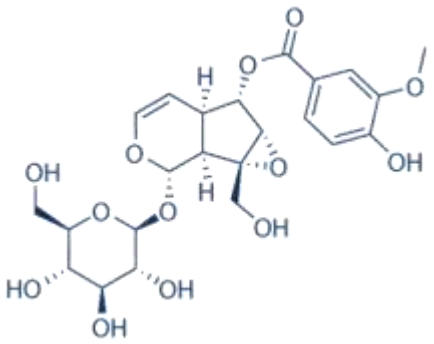

Figure 1: Chemical Structure of Picroside-II

Traditionally, *P. kurroa* has been used in Ayurvedic medicine to treat liver disorders[4,5], fever, asthma[5], gastrointestinal and urinary conditions. PK-II exhibits multiple pharmacological activities, including hepatoprotective, neuroprotective[6,7], anticancer, anti-inflammatory[8], and antioxidant effects[9]. Past research has focused on estimating PK-II in *P. kurroa* rhizome extracts using UV spectrophotometric methods[10], and various chromatographic methods are also available for its analysis[11]. However, no UV spectrophotometric methods have been developed for quantifying PK-II in formulated products using green or aqueous solvent. Addressing this gap, and recognizing the growing commercial significance of herbal products, a simple, precise, accurate, and cost-effective UV-Visible spectrophotometric method has been developed and validated for PK-II estimation in proliposome and herbal pharmaceutical formulations.

EXPERIMENTAL

MATERIAL USED

Pircoside-II was purchased from TCI Chemicals, India. Kurki Ghana Tablets (Chaitanya Pharmaceuticals) were purchased from local ayurvedic store of Chhatrapati Sambhajinagar, India. HPLC grade water was obtained from water purification system (Lablink, India). All the other chemicals used for the proposed were of analytical grade.

INSTRUMENTS USED

A double-beam Jasco UV-Visible spectrophotometer (UV-530 Jasco) equipped with Spectra Manager software was used for the analysis. A quartz cuvette with a 3 cm length and 1 cm path length was employed. An analytical weighing balance (Essae Vibra) with internal calibration mode was used for accurate weighing.

SOLVENT SELECTION

The solubility of PK-II was studied in various organic solvents including methanol, ethanol and deionized water. Based on the practical and theoretical solubility data, deionized water was selected for method development [3]

PREPARATION OF STANDARD STOCK SOLUTION

Ten mg PK-II was accurately weighed and dissolved in 10 mL of deionized water (DW) so as to achieve a Stock-1 solution of 1 mg/ml. A Stock-1 solution was diluted suitably so as to achieve stock-2 solution of 100 µg/mL strength.

DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORBANCE (λ_{MAX})

Stock-2 solution was diluted appropriately to achieve PK-II solution of 10 µg/mL strength. Said solution was scanned over the entire UV-Visible range of 200 to 800 nm using UV-Visible spectrophotometer with medium scanning speed. Scanning was performed using water as a blank. The wavelength of maximum absorbance (λ_{max}) was determined using software. To ensure reproducibility, above mentioned procedure was repeated five times.

PREPARATION OF CALIBRATION CURVE

To prepare a calibration curve, nine calibration standards (CAL STD) were prepared by diluting Stock-2 solution to so as to achieve CAL STDs of 1, 2 , 3, 5, 7, 10, 15, 20, 25 and 30 µg/mL strength. The absorbance of each CAL STD was measured at a predetermined wavelength of maximum absorbance of 218 nm. A concentration versus absorbance graph was plotted. To ensure reproducibility, the procedure was performed thrice.

METHOD VALIDATION

The developed UV method for estimating PK-II was validated in accordance with the ICH Q2(R1) guidelines. Various parameters, including linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness were evaluated.

Linearity and Range

The linearity and range of the proposed UV method were established using nine calibration standards. Each calibration standard was analysed for its absorbance at 218 nm and concentration vs. absorbance graph was plotted.

The calibration curve was subjected to linear least squares regression analysis, with the R-square (R^2) value serving as a key indicator for confirming the linearity of the method. The range of the proposed UV method was defined as the interval between the highest and lowest concentrations that demonstrated acceptable linearity.

Accuracy

The accuracy of the proposed UV-Visible spectrophotometric method was evaluated using the percent difference (% Difference). Three quality control (QC) standards—low quality control (LQC), medium quality control (MQC), and high-quality control (HQC)—with nominal concentrations of 2 µg/mL, 12 µg/mL, and 28 µg/mL, respectively were selected for accuracy determination. Each QC standard was prepared in pentaplicate to assess repeatability. The predefined QC standards were analysed for PK-II content three times a day (intra-day) and over the three consecutive days (inter-day). The absorbance of each QC standard was recorded, and the mean measured concentration was calculated. The accuracy of the method was determined using the following formula for % Difference.

$$\%Difference = \frac{(Mean\ Measured\ Concentration - Nomminal\ Concentration)}{Nominal\ Concentration} X\ 100$$

Precession

The precision of the proposed UV-Visible spectrophotometric method was evaluated through intra-day and inter-day analyses of predefined LQC, MQC and HQC standards. For the intra-day precision study, three PK-II solutions at concentrations of 2 µg/mL, 12 µg/mL, and 28 µg/mL were prepared in pentaplicate and analyzed in the morning, afternoon, and evening of the same day. Similarly, the inter-day precision study was conducted by repeating the same procedure on the three consecutive days. The variability of the results was calculated as the percentage relative standard deviation (%RSD). The following formula was used to determine the precession of the method.

$$\%RSD = \frac{Standard\ Deviation\ (SD)}{Mean\ Measured\ Concentration} X\ 100$$

Robustness

The robustness of the proposed UV-Visible spectrophotometric method was demonstrated by altering the wavelength. The wavelength was changed by ±1 nm, and the LQC, MQC and HQC standards were scanned for its PK-II content in

triplicate at 217 nm and 219 nm. The concentration of Picroside-II (PK-II) in each sample was estimated using the respective calibration curve, and the results were expressed in terms of percentage relative standard deviation (%RSD).

Ruggedness

The ruggedness of the developed UV method was depicted by analyzing LQC, MQC and HQC standards in triplicate on different UV-visible spectrophotometers. The concentration of PK-II in each sample was estimated using the respective calibration curve, and the results were expressed in terms of percentage relative standard deviation (%RSD).

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

The LOD and LOQ of the proposed UV-Visible spectrophotometer was calculated using following formula.

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

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

Where,
σ = Standard deviation of the y-intercept of regression line
S = average slope of the calibration curve

APPLICATION OF THE PROPOSED UV-VISIBLE SPECTROPHOTOMETRIC METHOD OF PK-II

Estimation of PK-II in its in-house Proliposomal formulation

The PK-II content in the in-house developed Proliposomes was calculated by an indirect method. Accurately weighed 10 mg of PK-II proliposomes were transferred to micro-centrifuge tubes and in each centrifuge tube, 2 mL of deionized water (DW) was added and the tubes were subjected to vortex mixing for the two minutes. After vortex mixing, samples were centrifuged at 14,000 rpm for 20 minutes. One mL supernatant of each micro-centrifuge tube was transferred to volumetric flask (5mL capacity) and diluted to 5 mL using deionized water. Samples were analysed for PK-II content using proposed UV-Visible spectrophotometric method. The calculated amount of PK-II was designated as un-entrapped PK-II. The difference between the actual PK-II amount added in the Proliposomes and the calculated un-entrapped PK-II was reported to be the PK-II content of the in-house proliposomes. The PK-II estimation experiments were repeated five times so as to achieve the accurate results.

Estimation of PK-II in Picrorhiza kurroa Extract formulation

Twenty tablets of the three different marketed formulation of *Picrorrhiza kurroa* extract were triturated separately using a mortar and pestle. A 10 mg of the powdered tablets was placed separately in a 1 mL micro-centrifuge tube and diluted with 1 mL of deionized water (DW), followed by vertexing for 30 seconds. The samples were then centrifuged at 14,000 rpm for 15 minutes to settle undissolved fine particles. Suitable dilutions were prepared and the samples were analyzed using a proposed UV spectrophotometric method to determine the concentration of PK-II. The PK-II estimation experiments were repeated five times so as to achieve the accurate results.

RESULT AND DISCUSSION

WAVELENGTH SELECTION

The sensitivity of the developed UV method is influenced by the choice of wavelength, as it

directly affects the method's ability to detect and quantify the analyte accurately. To determine the wavelength of maximum absorbance, PK-II solution of 10 µg/ml strength was scanned over the range of 200 to 800 nm. The wavelength of maximum absorbance for PK-II was found to be 218 nm.

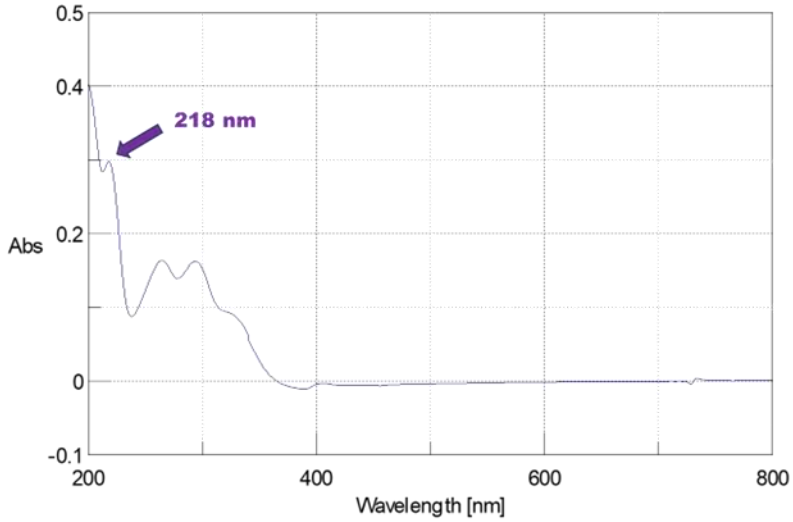

Figure 2: UV Visible Spectra of Picroside-II

PREPARATION OF CALIBRATION CURVE

The calibration curve represents the relationship between known concentrations and their corresponding responses. The equation derived from the calibration curve provides a mathematical model of the relationship between concentration and its responses viz. absorbance. In order to derive the reliable relation between concentration and absorbance, nine distinct CAL STDs were used. The calibration standards and the corresponding responses ± SD are shown in Table No. 1.

Table 1: Calibration standard data of PK-II

Concentration (µg/ml)	Absorbance ± SD
1	0.0359 ± 0.0024
3	0.0939 ± 0.0080
5	0.1699 ± 0.0376
7	0.2133 ± 0.0021
10	0.2915 ± 0.0245
15	0.4688 ± 0.0062
20	0.6113 ± 0.0096
25	0.7649 ± 0.0036
30	0.8986 ± 0.0102

VALIDATION

The validation of the developed UV-Visible spectrophotometric method was conducted to ensure its reliability, accuracy, and suitability for its intended purpose. The ICH Q2(R1) guideline is widely used in academia and industry for method validation. Accordingly, the developed UV method for quantifying PK-II in proliposome and herbal formulations was validated in accordance with the ICH Q2(R1) guidelines. The results are presented and discussed below.

Linearity and Range

PK-II demonstrated excellent linearity across a concentration range of 1 to 30 µg/mL, as determined by the calibration curve constructed from nine calibration standards. The linear least-squares regression analysis yielded a coefficient of determination (R^2) value of 0.999 with consistent slope and y-intercepts, indicating a highly linear relationship between concentration and absorbance, confirming the reliability and

suitability of the UV method for quantifying PK-II within this range. Linearity and Range illustrated in Figure 3.

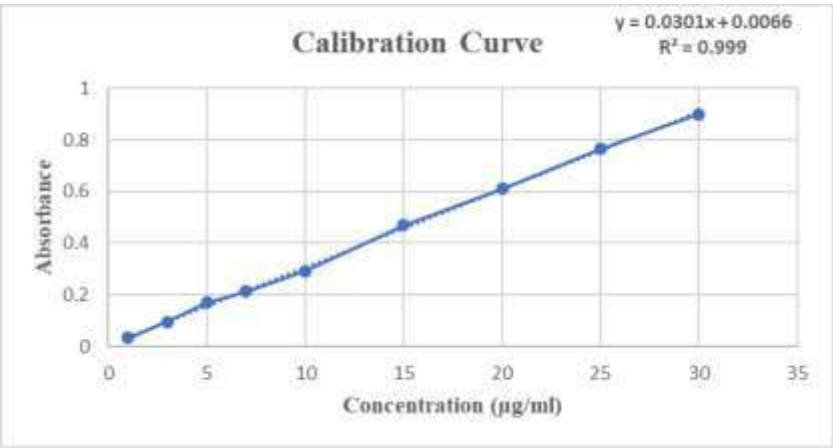

Figure 3(A): Calibration Curve for PK-II

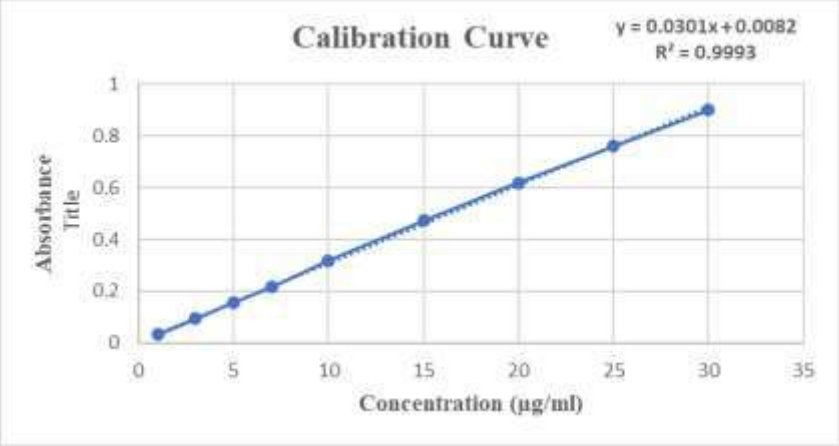

Figure 3(B): Calibration Curve for PK-II

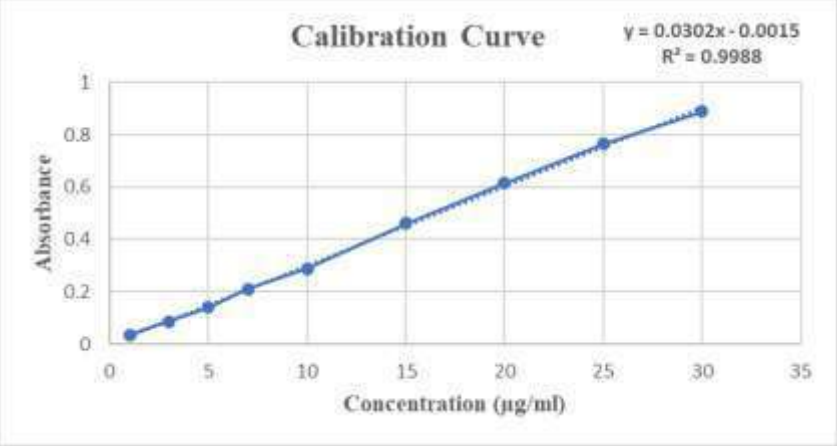

Figure 3(C): Calibration Curve for PK-II

Accuracy

The accuracy of a method is defined as the closeness of its results to the true or accepted

reference value. Accuracy was determined by calculating the percent difference (% Difference). The intra-day and inter-day % Difference values are presented in Tables 2 and 3, respectively. The intra-day accuracy ranged from -0.03 to 0.12, while the inter-day accuracy ranged from 0.04 to 0.4. Based on these results, the developed UV-Visible spectrophotometric method of PK-II was found to be accurate.

Table 2: Evaluation data of Intra-day Accuracy

Concentration Level	Nominal Concentration (µg /ml)	Mean Measured Concentration (µg /ml)	% Difference
LQC	2	2.11	0.05
	2	2.24	0.12
	2	1.94	-0.03
MQC	12	12.18	0.02
	12	12.10	0.01
	12	12.53	0.04
HQC	28	28.40	0.01
	28	28.38	0.01
	28	28.85	0.03

Table 3: Evaluation data of Inter-day Accuracy

Concentration Level	Nominal Concentration (µg /ml)	Mean Measured Concentration (µg /ml)	% Difference
LQC	2	2.24	0.12
	2	2.10	0.05
	2	2.79	0.40
MQC	12	12.53	0.04
	12	12.53	0.04
	12	12.16	0.01
HQC	28	28.42	0.02
	28	28.85	0.03
	28	28.44	0.02

Precision

Precision reflects the consistency with which a method produces the same results upon repeated measurements. Precision was assessed by calculating the percentage relative standard deviation (%RSD). The intra-day and inter-day precision values of UV-Visible spectrophotometric method of PK-II are presented in Tables 4 and 5, respectively. The intra-day %RSD values were found to be in between 0.8029 to 1.3888 whereas the inter-day %RSD values were found to be in between 0.6370 to 1.9202. The low %RSD values (<2) indicated that the proposed UV-Visible spectrophotometric method of PK-II is precise.

Table 4: Evaluation data of intra-day precision study

Concentration range (µg/ml)	Morning			Afternoon			Evening		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
2	2.1122	0.021	0.9972	2.2458	0.0431	1.9202	1.9415	0.0269	1.3880
12	12.1853	0.2948	2.4199	12.1601	0.0927	0.7628	12.5355	0.1488	1.1872
28	28.4	0.3172	1.1172	30.3827	0.1330	1.3344	28.8544	0.2316	0.8029

Table 5: Evaluation data of inter-day precision study

Concentration range (µg/ml)	Day 1			Day 2			Day 3		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
2	1.9415	0.0269	1.388	1.941	0.0269	1.3880	2.2458	0.0431	1.9202
12	12.5355	0.1488	1.1872	12.5355	0.1488	1.1872	12.1601	12.1601	0.7628
28	28.4	0.3172	1.1172	28.85	0.2316	0.8029	28.4478	28.4478	0.6370

Robustness

The robustness of an analytical method refers to its ability to remain unaffected by small, deliberate variations in method parameters, ensuring reliable results during routine analysis. To demonstrate the robustness of the developed UV-Visible spectrophotometric method, a deliberate change of ±1 nm in the absorbance wavelength was introduced. The proposed quality control (QC) standards were analysed in triplicate for the robustness study. The percentage relative standard deviation (%RSD) was found to be below 2%. These deliberate variations in the detection wavelength showed no significant change in absorbance, confirming the method's robustness. The robustness study data for the developed UV-Visible spectrophotometric method are presented in Table 6.

Table 6: Evaluation data of Robustness study

Concentration (µg /ml)	Wavelength (nm)	Mean Concentration	RSD	%RSD
2	217	2.061453	0.000361	0.428213
12	217	12.08101	0.002425	0.547499
28	217	27.69832	0.014731	1.470152
2	219	2.226257	0.001000	1.109878
12	219	12.28957	0.000643	0.142753
28	219	28.08939	0.010440	1.027589

Ruggedness

Ruggedness refers to a method's ability to produce consistent results despite minor variations in external experimental conditions or laboratory settings. To evaluate the ruggedness of the proposed UV-Visible spectrophotometric method, a different UV-Visible spectrophotometer in a distinct laboratory setting was used for the analysis. The proposed quality control (QC) standards were analysed in triplicate for the ruggedness study. The percentage relative standard deviation (%RSD) was found to be below 2%, indicating no significant difference in analyte concentration across repeated measurements. These deliberate variations in analytical instruments showed no significant change in absorbance, confirming the method's ruggedness. The ruggedness data are presented in Table 7.

Table 7: Evaluation data of Ruggedness

Concentration (µg /ml)	Mean Absorbance	RSD	%RSD
2	2.255121	0.001079	1.183518
12	12.81192	0.003769	0.803453
28	27.42551	0.001973	0.19886

Limit of detection and quantification

The LOD is the lowest amount of analyte that can be detected, though not necessarily quantified, while the LOQ is the lowest amount that can be quantified with acceptable precision and accuracy. The LOD and LOQ for PK-II were calculated using the formula mentioned earlier. The LOD was found to be 0.6470 µg/mL, and the LOQ was 1.9606 µg/mL. These low values demonstrated that proposed UV-Visible spectrophotometric method for PK-II is sensitive. Table 8 represent the LOD and LOQ data.

Table 8: Evaluation data for LOD and LOQ

LOD	0.6470 µg/ml
LOQ	1.9606 µg/ml

APPLICATION OF THE PROPOSED UV-VISIBLE SPECTROPHOTOMETRIC METHOD OF PK-II

Estimation of PK-II in its in-house Proliposomal formulation

The proposed UV-Visible spectrophotometric method was successfully utilized for the quantification of PK-II in proliposomal formulation. The estimated content of PK-II in the Proliposomes by the proposed UV-Visible spectrophotometric method is shown in Table No. 9.

Table 9: PK-II content in its in-house Proliposomes

Sr.	Formulation	Theoretical Content of PK-II (mg)	Practical content of PK-II (mg ± S.D.)
1.	Proliposomes	3.87	3.82 ± 0.21

(n = 5)

By using the proposed, Analysis revealed that 7.08 mg of PK-II was present externally to the proliposomes, while 3.87 mg was encapsulated within the formulation.

Estimation of PK-II in Picrorhiza kurroa Extract formulation

The proposed UV-Visible spectrophotometric method was successfully utilized for the quantification of PK-II in three different marketed, commercial formulations of *Picrorhiza kurroa* Extract. The PK-II content of the said formulations is shown in Table No. 10.

Table 10. PK-II content in marketed *Picrorhiza kurroa* Extract formulations

Sr.	Formulation	Practical content of PK-II (mg ± S.D.) per 10 mg formulation
1.	Formulation-A	0.497 ± 0.018
2.	Formulation-B	0.511 ± 0.019
3.	Formulation-C	0.523 ± 0.021

(n = 5)

CONCLUSION

A simple, validated UV-Visible spectrophotometric method was developed for the quantitative estimation of PK-II in its in-house Proliposomes and the marketed, commercial formulations. The method exhibited high accuracy and precision along with excellent robustness and ruggedness. It was successfully applied for accurate determination of PK-II content in both proliposomal and marketed tablet formulations.

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