DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PICROSIDE I AND PICROSIDE II IN BULK DRUG AND PHARMACEUTICAL FORMULATION

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ABSTRACT

To develop and validate a simple, precise, accurate, and sensitive UV-visible spectrophotometric method for the simultaneous estimation of Picroside I and Picroside II in a bulk drug and pharmaceutical formulation according to the ICH guidelines.

Methods: The absorption spectra of Picroside I and Picroside II were carried out over the range of 200-800 nm, and absorption maxima were determined. Multiple calibration standards were prepared of both the drugs separately, and absorbances were recorded at respective λmax. Calibration curve were plotted and the linear responses were studied. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness and ruggedness were calculated using QC standards.

Results: The absorption maxima of Picroside I and Picroside II were found to be 281 nm and 265 nm respectively. Linearity range for Picroside I and Picroside II were found to be 1-10 µg/ml and 1-40 µg/ml with correlation coefficient 0.999 and 0.999. The intra-day

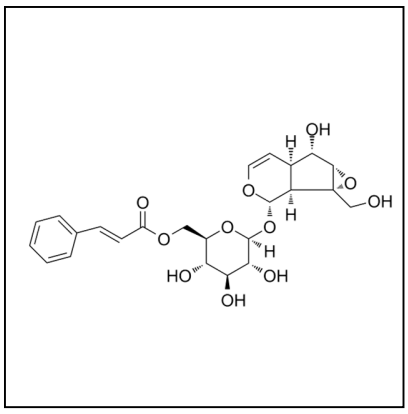
study for Picroside I and Picroside II shows percent relative standard deviation in the range of 0.061 to 0.769 and 0.054 to 0.862 and inter-day study for Picroside I and Picroside II shows percent relative standard deviation in the range of 0.026 to 0.950 and 0.073 and 0.8028. LOD and LOQ were found to be 0.3048 µg/ml and 0.9237 µg/ml for Picroside I whereas 0.2876 µg/ml and 0.8715µg/ml for Picroside II. The total percent recovery of Picroside I and Picroside II were found to be 99.057% to 100.61% for Picroside I and 99.387% to 100.61% respectively.

Conclusion: The simple, precise, accurate, and sensitive UV-visible spectrophotometric method for the simultaneous estimation of Picroside I and Picroside II in a bulk drug and pharmaceutical formulation was developed and validated.

Keywords: UV-visible spectrophotometry, Simultaneous estimation, Picroside I and Picroside II

Introduction

Picrorhiza kurroa is also commonly known as Kutaka or Kutki which is belonging to family Plantaginaceae. It is a perennial herb and is used as a substitute for Indian gentian (Gentiana kurroo).[1-3] The rhizome of this plant has a long history of use in Indian Ayurvedic medicine for the treatment of digestive problems and other disease conditions.[4-7] Rhizome of Picrorhiza kurroa plant contains chemical compositions such as Picroside I, Picroside II, d-mannitol, Kutkiol, Kutki Sterol, Picroliv and Apocynin in which Picroside I and Picroside II are one of the vital phytochemicals present in this plant. Picroside I is chemically known as [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[[(1S,2S,4S,5S,6R,10S)-5-hydroxy-2-(hydroxymethyl)-3,9-dioxatricyclo[4.4.0.$0^{2,4}$]dec-7-en-10-yl]oxy]oxan-2-yl]methyl (E)-3-phenylprop-2-enoate and that of Picroside II is chemically known as [(1S,2S,4S,5S,6R,10S)-2-(hydroxymethyl)-10-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,9-dioxatricyclo[4.4.0.$0^{2,4}$]dec-7-en-5-yl] 4-hydroxy-3-methoxybenzoate.[8-11]



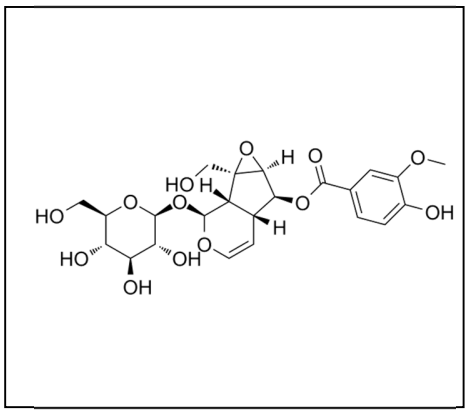


Fig.1. Chemical structure of Picroside I **Fig.2. Chemical structure of Picroside II**

Picroside I and Picroside II are widely used to treat asthma, liver damage, wound healing, and vitiligo. It is used to treat all types of liver damage, cirrhosis, and liver inflammation. It protects the liver from hepatitis C viral damage. Picroside I and Picroside II have been shown to have a variety of pharmacological actions, including hepatoprotective, anti-inflammatory, and neuroprotective properties. The anti-cancer effects of Picroside I and Picroside II on cancer cells, however, are unknown.[(10-15)]Picroside I and II are mostly soluble in organic solvents such as Methanol ,ethanol ,dimethyl sulfoxide, and poorely soluble in water . Picroside I has a partition coefficient of -1.1, while Picroside II has a partition coefficient of -1.4.Inspite of well-established physicochemical and therapeutic importance, there is no any analytical technique was developed on the combination of Picroside I and Picroside II have been reported across the world. Till date, there is no single UV Visible Spectrophotometric method available for accurate quantification of Picroside I and Picroside II from the plant Picrorhiza kurroa particularly as per Simultaneous Estimation equation.

Considering the future potential of Picroside I and Picroside II, an accurate, precise and cost-effective UV Visible spectrophotometric method was developed and validate. Developed method was successfully used for the estimation of Picroside I and Picroside II in the various extract of Picrorhiza kurroa.

MATERIALS AND METHODS

Materials

Picroside I and Picroside II (purity 98% by HPLC) was obtained as a gift sample from natural product chemistry, Division of Indian Institute of Integrative Medicine (CSIR), Jammu. Methanol was purchased from Merck. All the chemicals of analytical grade were used for are used for the proposed study.

Instruments Used

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

Preparation of standard stock solution

The standard stock solution having concentration 1000 µg/ml(Stock-I) of each Picroside I and Picroside II were prepared separately by dissolving accurately weighed 5 mg of API in 5 ml Methanol. Stock-I solution of both the bulk drug were further suitably diluted with solvent system methanol to achieve the solution of concentration 100 µg/ml (Stock-II) and 10 µg/ml (Stock-III).Similarly, the standard stock solutions of combined dosage form of Picroside I and Picroside II were prepared having the concentrations 1000 µg/ml, 100 µg/ml and 10 µg/ml.

Determination of wavelength of maximum absorbance (λmax)

The standard stock solution of Picroside I and Picroside II with a concentration of 10µg/ml (Stock-III) was scanned individually against the reference sample Methanol in the UV region of 200-800 nm and the spectra was recorded. Both bulk medicines maximum wavelength were determined. The aforementioned procedure was performed 5 times to ensure correctness.

Preparation of calibration curve

Calibration curve were defined by diluting the stock-II standard solution of both bulk drug i.e. Picroside I and Picroside II to achieve the seven different calibration standards i.e. CAL STD - 1 (1µg/ml), CAL STD - 2 (2µg/ml), CAL STD - 3 (3µg/ml),CAL STD - 4 (4µg/ml),CAL STD - 5 (6µg/ml),CAL STD - 6 (8µg/ml) and CAL STD - 7(10µg/ml) for Picroside I and CAL STD - 1 (1µg/ml), CAL STD - 2 (5µg/ml), CAL STD - 3(10µg/ml), CAL STD - 4 (15µg/ml), CAL STD - 5 (20µg/ml), CAL STD – 6 (30µg/ml), CAL STD – 7 (40µg/ml) for Picroside II. Using fixed wavelength measurement mode, each calibration standard was scanned at pre-defined maximum wavelengths of 281 nm and 265 nm for Picroside I and Picroside II, respectively. The absorbance at respective wavelength were noted of various calibration standards. Separately, the concentration vs. absorbance graph was created using Microsoft Office 2010's Excel program. To achieve reproducible results, the procedure was repeated five times.

Simultaneous Equation method OR Vierodt's method

To determine both drugs by the technique of simultaneous equation method (vierodt'smethod), sample should contain two absorbing drugs each of which absorbs at the λmax different from the other. The two absorbing drug Picroside I and Picroside II Each of which absorbs at the λ max of the other, then both the drugs can be quantified by using simultaneous equation method.

Determination of absorptivity value

The absorptivity of each solution was calculated by using the following formula:

Absorptivity = Absorbance/Concentration (µg/ml).

The concentration of both the drug can be obtained by formula -

Cx = (A2ay1 – A1ay2) / (ax2ay1 - ax1ay2) …..I

Cy = (A1ax2– A2ax1) / (ax2ay1 - ax1ay2) ……II

Where,

λ1: Wavelength maxima for Picroside I

λ2: Wavelength maxima for Picroside II

ax_1 and ax_2: Absorptivity of Picroside I 281nm and 265 nm

ay_1 and ay_2: Absorptivity of Picroside II 281 nm and 265 nm

A1: Absorbance of Picroside I at 281 nm

A2: Absorbance of Picroside II at 265 nm

CX and CY: the concentration of Picroside I and Picroside II respectively in the diluted sample

METHOD VALIDATION

The developed UV method for the estimation of Picroside I and Picroside II in bulk drug and pharmaceutical formulation was validated as per the ICH guidelines. Various parameters like linearity and range, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) were analyzed using pre-defined calibration standards or quality control standards as described below [15-16].

Linearity and Range

Linearity was evaluated by linear regression analysis and calculated by least square method. The calibration curves shows correlation between absorbance and concentration level within the concentration range of 1-10µg/ml for Picroside I and 1-40 µg/ml for Picroside II. Plots were subjected to linear regression least square analysis. R^2 value was important factor for establishing linearity. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy was determined by means of recovery studies by evaluating % mean recovery of both the drugs.The known concentrations of drug were added at the different level viz. 80%, 100%, and 120% level. The pentaplicate of three different pre-defined concentration solutions of both the drugs i.e. Picroside I(1.5, 5, and 9.5 µg/ml) and Picroside II(1.5, 22,and 39µg/ml)were prepared. The absorbance were measured at wavelength 281 nm and 265 nm wavelength for Picroside I and Picroside II respectively. The above method was perform five times.

The percent recovery was calculated by formula -

%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 repeatability of method was checked by statistical evaluation. Intraday and inter day variations were studied. Five different solution of both the drug were prepared for Picroside I(LQC-1.5µg/ml, MQC-5µg/ml, and HQC-9.5 µg/ml) and Picroside II(LQC-1.5µg/ml, MQC-22µg/ml, and HQC-39µg/ml) and analyzed at morning, afternoon and evening time of three consecutive days. Deviations in the results were calculated in terms of % RSD (% relative standard deviation).

Robustness

Robustness was determined by changing the wavelength ±1nm from 281nm for Picroside I and 265nm for Picroside II. Middle level quality control sample of Picroside I (5µg/ml) and Picroside II (22µg/ml) was prepared and analyzed at pre-defined wavelength. The results were calculated in terms of % RSD.

Ruggedness

Ruggedness study of the method was carried out by analyzing triplicate samples of Picroside I (5µg/ml) and Picroside II (22 µg/ml) using two different instruments (V-530, Jasco and BA-UV-2600, Bioage) and Analysts. Results were expressed in terms of % RSD.

Limit of Detection (LOD)

The LOD of the developed UV method was determined by formula -

$LOD=3.3\times SD/S$

Where,

SD= Standard deviation of Y-intercepts

S= Slope of calibration curve

Limit of Quantification (LOQ)

The LOQ of the developed UV method was determined by formula -

$LOQ= 10\times SD/S$

Where,

SD= Standard deviation of Y-intercepts

S= Slope of calibration curve

RESULTS AND DISCUSSION

Method development

Picroside I and Picroside II are highly soluble in methanol .Hence it is used as a solvent for standard and sample preparation the use of Vierordt's or SE method allowed these drugs to be determined simultaneously. The absorbance values were taken at 281 nm and 265 nm for Picroside I and 265 nm and 281 nm for Picroside II respectively and then the calibration curves were plotted at each wavelength for both the drugs as presented in the fig 7,8, 9, 10 and their absorptivity values were calculated for each concentration.

Determination of wavelength of maximum absorbance (λmax)

Identification of wavelength having maximum absorbance is prerequisite for quantitative UV analysis. Solution with absorbance value less than 1 were considered to be appropriate for the determination of wavelength having maximum absorbance. Considering the above mentioned point determination of λmax of Picroside I and Picroside II solution of 10 µg/ml concentration each were carried out by full scan mode of UV-Visible spectrophotometer. The full scan mode was processed by Jasco UV software and λmax were determined. The λmax was found to be 281nm and 265nm for PicrosideIandPicroside II (Fig. 3 and Fig. 4) respectively.

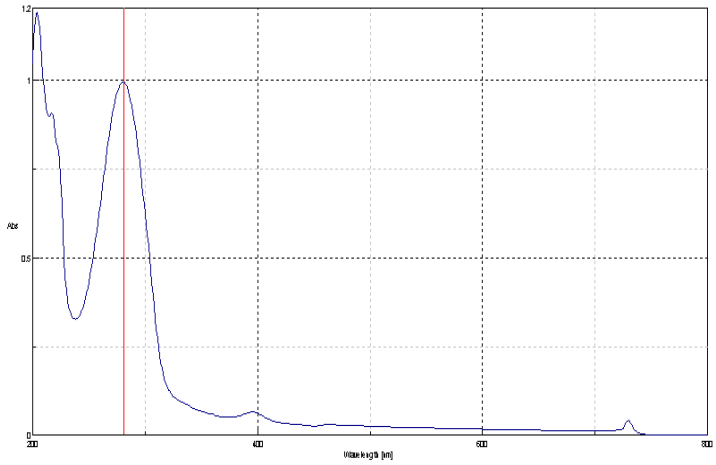


Fig. 3. Picroside I Peak at 281nm

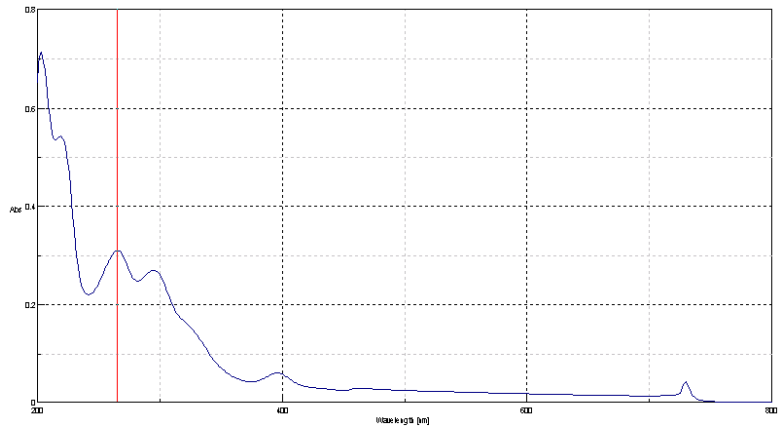


Fig.4. Picroside – II at 265nm

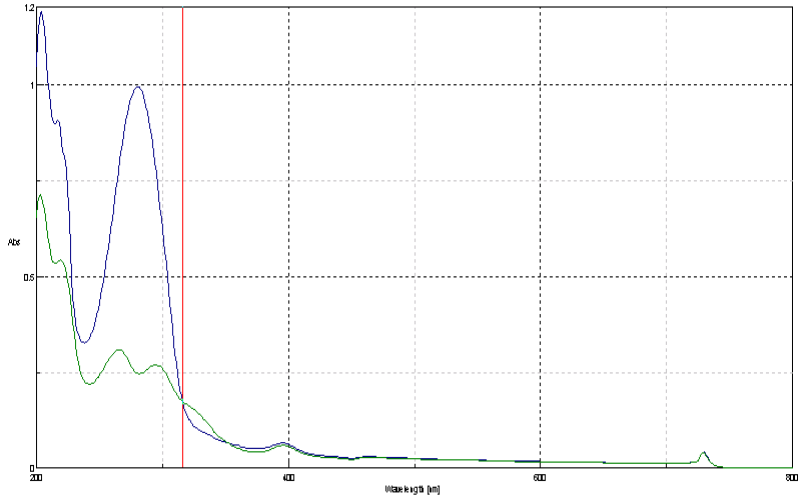


Fig. 5 – Overlap spectra of Picroside I and Picroside II

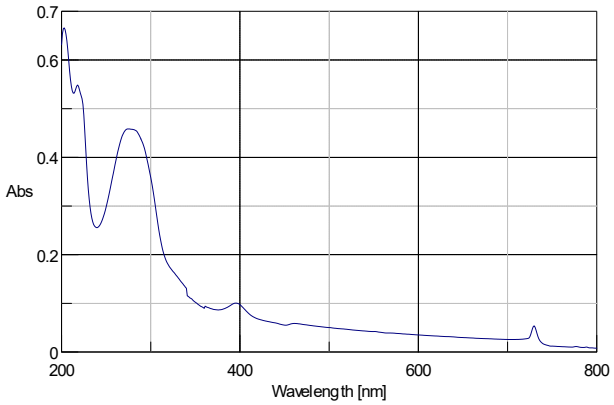


Fig.6- UV spectra of mixture containing Picroside I and Picroside II

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis requires reproducible calibration curve and a mathematical equation representing correlation between concentration and the response. Considering the utility of quantitative analysis of Picroside I and Picroside II, calibration curve for both drug were developed using seven different calibration standards. The absorbances of different calibration standards at wavelength 281 nm and 265 nm for Picroside I and Picroside II respectively were recorded by fixed wavelength mode. Calibration curve was repeated five times.

Table 1. Linearity study for Picroside I and Picroside II

Sr. No.	Conc. (µg/ml)	Absorbance of Picroside I at 281 nm	Conc. (µg/ml)	Absorbance of Picroside II at 265 nm
1	1	0.09982±0.0015	1	0.0259±0.0017
2	2	0.19956±0.0021	5	0.137±0.0020
3	3	0.29812±0.0019	10	0.243±0.0015
4	4	0.39865±0.0017	15	0.3606±0.0019
5	6	0.59915±0.0009	20	0.4896±0.0014
6	8	0.79956±0.0025	30	0.7352±0.0022
7	10	0.98224±0.0014	40	0.968±0.0026

Method validation

Linearity and range are the key parameters of analytical method which proposed the limit within the intended method to be used for its optimum performance. Considering the importance of linearity and the range, seven points calibration curve of Picroside I

between the range 1-10 µg/ml and Picroside II between the range 1-40 µg/ml were plotted. The concentrations and the respective mean absorbance values of Picroside I and Picroside II are mentioned in Table 2. Calibration curve for Picroside I and Picroside II with their respective wavelength i.e., 281nm and 265nm were subjected to least square regression analysis yielded an equation; y = 0.098x + 0.003 and y = 0.097x + 0.007with correlation coefficient and for Picroside I and Picroside II respectively (Fig. 6 and Fig. 7) and concentration of Picroside I and Picroside II with respect to their opposite wavelength that is for Picroside I wavelength is 265nm and for Picroside II wavelength is 281nm is y = 0.097x + 0.007 and y = 0.024x + 0.005 respectively (Fig.8 and Fig.9) .The linearity study revealed that the developed UV method was found to be linear adherence to the system of Beer's - Lambart's Law over the concentration range of 1 to 10 µg/ml for Picroside I and 1-40 µg/ml for Picroside II.

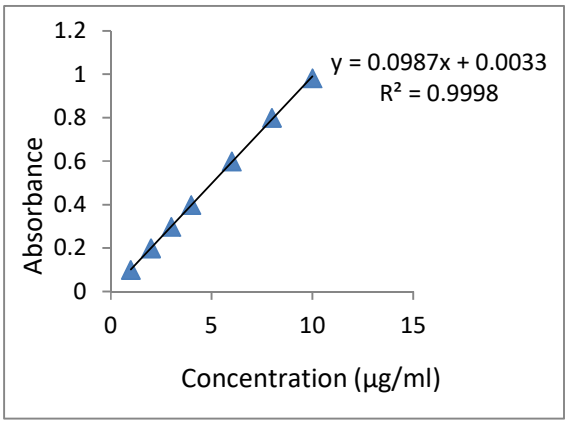


Fig 7.Calibration curve of picroside I at 281 nm

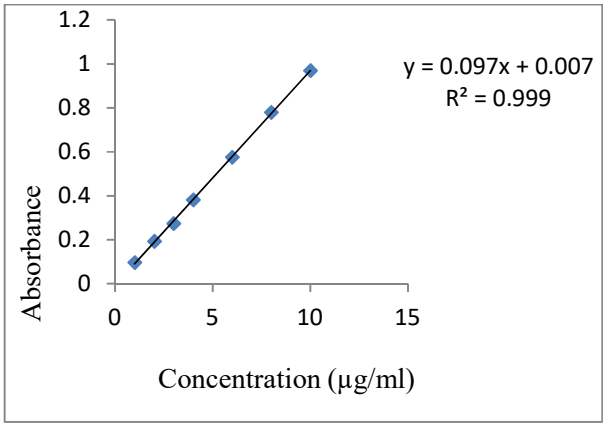


Fig.8: Calibration curve for Picroside I at 265nm

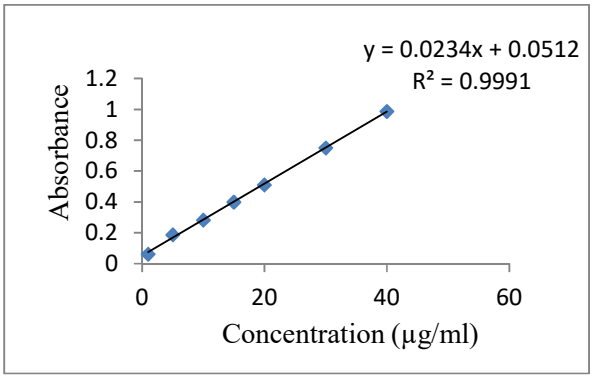


Fig.9: Calibration Curve of Picroside II at 265 nm

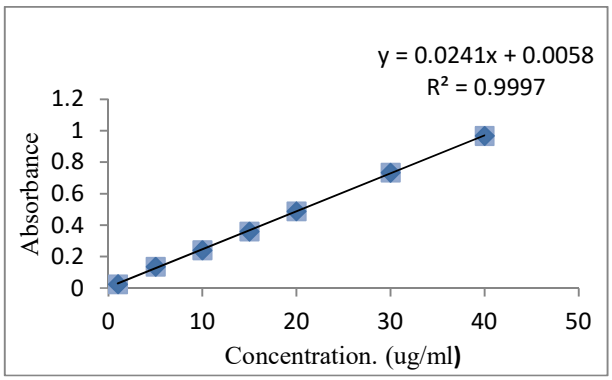


Fig.10: Calibration Curve of Picroside II at 281 nm

Table 2. Absorbance and Absorptivity Value For Picroside I and Picroside II

Conc. Of Solution (µg/ml)		Absorbance				Absorptivity			
		PK-I		PK-II		PK-I		PK-II	
PK-I	PK-II	281nm	265nm	281nm	265nm	281nm	265nm	281nm	265nm
1	1	0.0998	0.0955	0.0259	0.0622	0.0998	0.0955	0.0259	0.0622
2	5	0.1995	0.1926	0.1371	0.1876	0.0997	0.0963	0.0274	0.0375
3	10	0.2981	0.2735	0.2432	0.2836	0.0993	0.0945	0.0243	0.0283
4	15	0.3986	0.3816	0.3606	0.3998	0.0996	0.0929	0.024	0.0266
6	20	0.5991	0.5747	0.4896	0.5122	0.0998	0.0957	0.0244	0.0256
8	30	0.7995	0.7792	0.7352	0.7525	0.0999	0.0974	0.0245	0.0250
10	40	0.9822	0.9694	0.9682	0.9876	0.0982	0.0969	0.0242	0.0246
Avg.						Ax1=0.0991	Ax2=0.0956	Ay1=0.0249	Ay2=0.0328

Accuracy

Accuracy is the measure of 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. UV method for Picroside I and Picroside II, accuracy was established by recovery studies. Mean recovery of Picroside I was found to be 99.057, 100.61, and 100.42 and of Picroside II was found to be 99.387, 100.61, and 100.42 at 80 %, 100% and 120% standard addition respectively. %RSD were found to be less than 2 for the Picroside I and Picroside II, recovery studies are shown in Table 3. The results of

accuracy studies, determined that the developed UV method is highly accurate as the percent recovery was found to be between 99 to 100%.

Table 3. Recovery studies for Picroside I and Picroside II

Origin level (µg/ml)	PICROSIDE-1			Origin level (µg/ml)	PICROSIDE-2		
	Concentration (%)	% Recovery	% RSD		Concentration (%)	% Recovery	% RSD
1.5	80	99.057	1.174	1.5	80	99.387	0.6523
5	100	100.61	0.869	22	100	100.61	0.8692
9.5	120	100.42	1.128	39	120	100.42	1.128

Precision

Precision is a measure of degree of scatter, expresses the reproducibility of the measurements. It is expected that an analytical method should generate reproducible outcomes. Precise analytical method leads to accurate results. Considering the importance of reproducible and accurate results, intra-day and inter-day precision of developed UV method were established at LQC-1.5µg/ml, MQC-5µg/ml and HQC-9.5 µg/ml concentration levels of Picroside I and at LQC-1.5µg/ml, MQC-22µg/ml, and HQC-39 µg/ml concentration levels of Picroside II. The results were expressed in terms of mean absorbance values, percent assay and % RSD for the intra-day and inter-day precision study, demonstrated in Table 3 and Table 4 respectively for Picroside I and Picroside II. Percentage RSD values of intra-day precision study were found to be between 0.061 to

0.769 for Picroside I and between 0.054 to0.862 for Picroside II whereas those of inter-day precision study were between 0.026 to 0.950 for Picroside I and between 0.073 to 0.8028 for Picroside II. % RSD values were less than 2, demonstrated the precision of developed UV method

Table 4. Intra-day precision for Picroside I

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.50	100.45	0.448	1.51	101.11	0.769	1.52	101.57	0.601
5	5.05	101.03	0.171	5.04	100.9	0.254	5.06	101.33	0.233
9.5	9.49	99.92	0.061	9.49	99.98	0.022	9.46	99.61	0.113

Table 5. Intra-day precision for Picroside II

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.51	101.27	0.461	1.52	101.45	0.722	1..52	101.57	0.862
22	22.31	101.42	0.162	22.39	101.31	0.146	22.50	101.29	0.138
39	39.08	100.21	0.102	39.07	100.18	0.225	39.05	100.13	0.054

Table 6. Inter-day precision for Picroside I

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.49	99.83	0.075	1.52	101.38	0.794	1.529	101.93	0.950
5	5.06	101.38	0.196	5.05	101.01	0.166	5.04	100.87	0.295
9.5	9.44	99.46	0.091	9.50	100.02	0.060	9.50	100.04	0.026

Table 7. Inter-day precision for Picroside II

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.525	101.711	0.541	1.524	101.63	0.7028	1.514	100.96	0.802
22	22.310	101.411	0.190	22.301	101.36	0.175	22.277	101.2614	0.161
39	39.070	100.181	0.073	39.076	100.19	0.089	39.06	100.17	0.0746

Robustness

Robustness is the ability of a method to resist the change in its performance in spite of small un-intentional change in method parameters like solvent composition, buffer strength, pH, ±1nm wavelength etc. Change may occur and hamper the performance, it is expected that such change should not alter the performance of the analytical method. Hence, robust analytical method is studied. Robustness of proposed UV method was established by scanning the sample solution change in concentration of the mobile phase as well as change in scanning wavelength for ±1nm wavelength from 281nm for Picroside I and 265nm for Picroside II . Hence change in concentration of mobile phase

shows slight change in absorbance from 0.498 to 0.4983 (Table 8) for Picroside I and 0.5396 to 0.5403 (Table 9) for Picroside II as well as change in the wavelength by ±1nm did not as much affect the performance of developed method. The % RSD values were found to be between 0.1210 to0.4988 for Picroside I and between 0.1031 and 0.5401 for Picroside II, shown in (Table 10) for Picroside I and Picroside II respectively. % RSD values were below 2 depict that the proposed UV method was robust in nature.

Table 8. Robustness data of UV method for Picroside I and Picroside II

Concentration (µg/ml)	MeOH:Water Ratio	Absorbance	% RSD
5	49:51	0.4988	0.1304
5	50:50	0.4978	0.1210
5	51:49	0.4983	0.2126

Concentration (µg/ml)	MeOH:Water Ratio	Absorbance	% RSD
22	49-51	0.5401	0.1823
22	50-50	0.5399	0.2031
22	51-49	0.5400	0.1031

Table 10. Robustness data for Picroside I and Picroside II

Conc. (µg/ml)	Picroside I		Conc. (µg/ml)	Picroside II	
	λmax	Absorbance		Λmax	Absorbance
5	280	0.498	22	264	0.5396
5	282	0.4983	22	266	0.5403

Ruggedness

Ruggedness is the ability to resist the change in method performance in-spite of influential environmental factors like temperature, pressure, equipment, etc. Rugged analytical methods are free from environmental/external factors impact. The ruggedness

of proposed UV method, for Picroside I and Picroside II solutions were analyzed by using two different UV-Visible spectrophotometers belongs to different laboratories, analyst,etc. Sample analysis resulted into % RSD values between 0.1307 to 0.3420 for Picroside I and between 0.075 to 0.1297 for Picroside II. Results showed that the proposed UV method was rugged as % RSD values were less than 2, shown in Table 11 of Picroside I and Picroside II.

Table 11. Ruggedness study for Picroside I and Picroside II

Conc. (µg/ml)	Picroside I			Conc. (µg/ml)	Picroside II		
	Instrument	Absorbance	% RSD		Instrument	Absorbance	%RSD
5	Jasco	0.4975	0.1307	22	Jasco	0.5396	0.1297
5	Bioage	0.4970	0.2207	22	Bioage	0.5404	0.1203
5	Analyst I	0.4984	0.3420	22	Analyst I	0.5412	0.075
5	Analyst II	0.5031	0.2980	22	Analyst II	0.5438	0.115

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

Generally, LOQ is the first calibration standard. LOQ represents the lowermost concentration that can be analyzed. LOD represents the lowest quantity of substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). LOD and LOQ of proposed UV method were found to be 0.3048µg/ml to 0.9237µg/ml for Picroside I whereas 0.2876µg/ml to 0.8715µg/ml for Picroside II, as shown in Table 7 for Picroside I and Picroside II. Lower LOQ values indicated that the proposed method would be sensitive enough to quantify the Picroside I and Picroside II content of samples at its lower level.

Table 12. LOD and LOQ for Picroside I and Picroside II

Sr. No.	Parameter	Picroside I	Picroside II
1	LOD	0.3048µg /ml	0.2876µg/ml
2	LOQ	0.9237µg/ml	0.8715µg/ml

Estimation of Picroside I and Picroside II content in marketed pharmaceutical formulation

The developed UV method was successfully applied for estimation of Picroside I and Picroside II content in the marketed formulation Divya Kutki Churna (B.P.N.) Picroside I and Picroside II content in the powder was found to be 3% and 2.97% respectively by proposed method for simultaneous estimation of Picroside I and Picroside II.

CONCLUSION

The simple, precise, accurate, and sensitive UV- visible spectrophotometric method by the simultaneous estimation for Picroside I and Picroside II in Picrorhiza kurroa rhizome was developed and validated. Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of Picroside I and Picroside II present in Picrorhiza kurroa rhizome extracts.

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