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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE QUICK ESTIMATION OF METHANOLIC EXTRACT OF PICRORHIZA KURROA ROYAL EX. BENTH

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ABSTRACT

Picrorrhiza kurroa Royal ex. Benth. Belonging to the family Scrophulariaceae have been in Indian traditional medicine for centuries. Methanolic extract of rhizomes of Picrorrhiza kurroa has been obtained from continuous hot percolation process. Calibration curve of the rhizome extract was prepared in phosphate buffer of Ph 7.2 on two consecutive days at λ_{max} 357nm. The absorbance values with their standard deviations at different concentrations in the range of 10 to 180µg/ml. Extract was found to obey Beer-Lamberts law in the concentration range of 10-180µg/ml. The regression co-efficient (r^2) values 0.999. The regression equation was calculated as y=0.005x for phosphate buffer of Ph 7.2. The developed calibration curve was validated for intra-day and inter-day variations as per ICHQ2A guidelines and was to be found a stable method.

Keywords: Picrorrhiza kurroa, Continous hot percolation, Validation, UV Spectroscopy

INTRODUCTION

Picrorrhiza kurroa Royal ex. Benth. belonging to the family Scrophulariaceae is a small perennial herb that is widely distributed in the North-West India on the slopes of Himalayas between 3000 and 5000meters[1, 2].Picrorrhiza kurroa is well for its hepatoprotective, Anti-periodic, Cholagouge, stomachic, Anti-amoebic, Anti-oxidant, Anthelmintic, Anti-inflammatory, Cardio-tonic, Laxative, Carminative, Expectorant etc[3, 4].

There are no reported UV Visible methods for estimation of this extract which is necessary in the development of suitable formulations for this drug. Hence, a simple UV spectroscopic method was developed for direct estimation of this extract. The assay validation of calibration curve was carried out as per ICHQ2A guidelines. In validation procedure, calibration curve prepared in phosphate buffer of Ph 7.2 was run in duplicate for two days to determine intra-day and inter-day variations[5].

MATERIALS AND METHODS

Rhizomes of Picrorrhiza kurroa plant were procured from SV.University, Tirupati. All the other chemicals and reagents used in this study were of AR grade and were purchased from Qualikems fine chemicals, Sadar Bazar, New Delhi.

Methods

Collection and authentication of Rhizomes of Picrorrhiza kurroa Royal ex. Benth.

The plant specimen was collected from SV University, Tirupati, AP, India and has been identified as Picrorrhiza kurroa Royal ex. Benth belonging to the family Scrophulariaceae Voucher No- SDIP, Ref. No-002 dated 26/10/2012 and authenticated by Dr.K.Madhavachetty, Botanist, SV University, Tirupati, AP, India.

The rhizomes of the plant were cleaned and dried under ambient temperature and then in oven at 20-40°C. The dried rhizomes were weighed and stored in dessicator (1.3kgs).

Preparation of the plant extract

The extraction was done by continuous hot percolation using Soxhlet apparatus. The finely ground plant material (500gms) is placed in a porous bag or "thimble" made of strong filter paper, was packed in the column using adequate cotton on top and bottom of the plant material. The packed material is allowed to get moistened with adequate amount of menstrum and extraction has been carried out for 24hrs continuously. Later the extract that was obtained in round bottomed flask of soxhlet apparatus is subjected to distillation for separating the solvent for reuse. Than the extract is dried and stored in dessicator for further use.

DEVELOPMENT OF CALIBRATION CURVE

Selection of media

The selection of media was done on the basis of drug solubility phosphate buffer of Ph 7.2 was selected for the preparation of calibration curve[6].

Scanning for λ_{max}

50mg of crude drug was dissolved in little volume of phosphate buffer of Ph 7.2 and finally diluted to 50ml volumetric flask to get the concentration of 1000µg/ml. This was treated as stock solution. Various aliquots of stock solution were diluted further to get different concentrations. Resultant solutions were scanned for λ_{max} in the range of 200-400nm using double beam UV spectrometer Shimazdu- 1800.

Preparation of calibration curve

Aliquotes of the stock solution of rhizome extract (1000µg/ml) were pipette out into a series of 10ml volumetric flask and diluted with phosphate buffer of Ph 7.2 to get a final concentration of 10-180µg/ml. The absorbance of the resultant solutions was made for the calibration curve on 2 consecutive days.

Validation of calibration curve

Assay validation of the calibration curve was carried out as per USP guidelines in category I and as per ICHQ2A guidelines. In validation procedure, calibration curve prepared in phosphate buffer of Ph 7.2 was run in duplicate for 2 days to determine intra and inter day variations.

RESULTS AND DISCUSSION

The methanolic extract of rhizomes of Picrorrhiza kurroa Royal ex. Benth. was soluble in water, Phaosphate buffer of Ph 7.2, DMSO, ethanol and methanol and was insoluble in acetone, toluene, propanol, chloroform(Table-1). The λ_{max} of the drug in phosphate buffer of Ph 7.2 was determined using Shimazdu 1800 double beam UV spectrophotometer. The λ_{max} was determined by scanning 10µg/ml solution of drug in the test medium in the range of 200-400nm. The λ_{max} was found to be 357nm and the absorbance was found to be 0.051.

Calibrationn curve of drug was prepared in phosphate buffer of Ph 7.2 on 2 consecutive days at λ_{max} 357nm. The absorbance values with a standard deviation at different concentration in the range of

10-180µg/ml are given the Table-2. The calibration curve is given in fig-1. The extract was found to obey Beer-Lambert's law in the concentration range of 10-180µg/ml with a regression co-efficient (r^2) value 0.999. The regression equation was calculated as y=0.005x for phosphate buffer of Ph 7.2.

Accuracy can also be associated with the term bias. A biased estimate is systematically either higher or lower than the true value. Thus, for accuracy, recovery studies were carried out on the % recovery was found to be in the range of 99.45 – 99.75 which was within the recommended tolerance limit of 50-150%[7]. The results are shown in Table-3.

The precision of the analytical method or a test procedure is referred to as the degree of closeness of the result obtained by the analytical method or the test procedure to the true value. For evaluation of the precision, the RSD was determined and the range of RSD was 0.49 – 2.86, 0.49 – 2.21 for first and second days respectively in the intraday study, and was found to be in the range of 0.48 – 1.09 in the inter day assay. The results are given in the table 4-6. It is suggested that the analytical method may be considered validated in terms of precision if the precision around the mean value does not exceed 2.86% RSD.

Linearity of an analytical method is its ability to elicit test results that are directly, or by a well- defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. Linearity and range data for calibration curves prepared in phosphate buffer of Ph 7.2.

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantitated, under a stated experimental condition and the limit quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions. These two parameters are required for assay validation as per ICHQ2A guidelines. Limit of detection and Limit of quantitation of calibration curve were calculated which was based on the standard deviation of y-intercept of regression line (SD) and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD=3.3 (SD/S) and LOQ=10 (SD/S). LOD and LOQ of calibration curve of drug prepared in phosphate buffer of Ph 7.2. The results are given Table-7.

Table 1: Solubility profile of the Leaf extract of Picrorrhiza kurroa

Solvent	Solubility behaviour
Water	Soluble
Phosphate buffer, Ph 7.2	Soluble
DMSO	Soluble
Acetone	Insoluble
Toluene	Insoluble
Propranolol	Insoluble
Chloroform	Insoluble
Methanol	Soluble
Ethanol	Soluble

Table 2: Calibration curve data of the Leaf extract of Picrorrhiza kurroa in phosphate buffer Ph 7.2

Concentration (µg/ml)	Absorbance
10	0.051
20	0.101
40	0.203
60	0.306
80	0.413
100	0.515
120	0.617
150	0.770
180	0.918

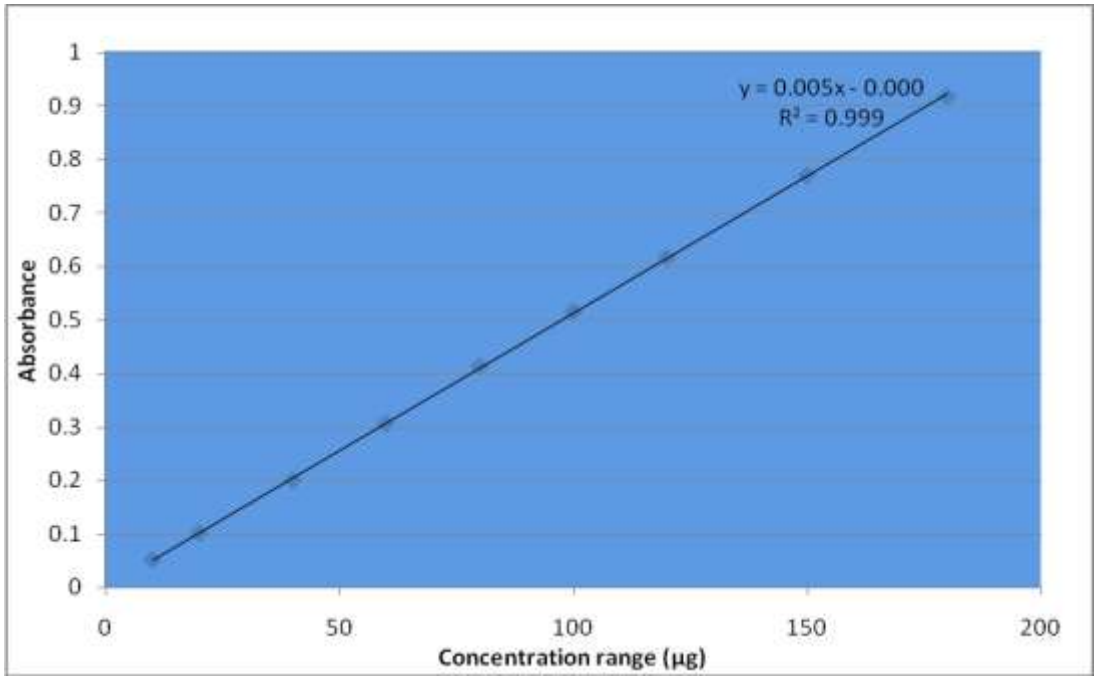

Fig. 1: Calibration curve data of Picrorrhiza kurroa extract in phosphate buffer Ph 7.2

Table 3: Recovery results of drug for determination of Accuracy

Labelled amount (µg)	Spike value (µg)	Amount recovered (µg)	Percentage recovery (%)
50	50	99.75	99.75
100	100	197.53	98.76
150	150	298.35	99.45

Table 4: Results of intraday precision studies for day one

Concentration (µg/ml)	Absorbance	Mean absorbance	SD	RSD
10	0.052 0.053 0.055	0.053	0.095	2.86
20	0.105 0.104 0.105	0.104	0.0005	0.55
40	0.202 0.201 0.199	0.200	0.0015	0.76
60	0.305 0.302 0.304	0.303	0.0015	0.50
80	0.412 0.413 0.416	0.413	0.0020	0.50
100	0.512 0.515 0.517	0.514	0.0025	0.49

Table 5: Results of intraday precision for day 2

Concentration (µg/ml)	Absorbance	Mean absorbance	SD	RSD
10	0.062 0.061 0.061	0.061	0.001	2.21
20	0.105 0.105 0.107	0.105	0.0011	1.13
40	0.203 0.205 0.202	0.202	0.001	0.77
60	0.316 0.313 0.315	0.314	0.002	0.82
80	0.418 0.417 0.408	0.414	0.002	0.61
100	0.518 0.515 0.508	0.513	0.002	0.49

Table 6: Results of interday precision studies of the calibration curve of Picrorrhiza kurroa extract

Concentration (µg/ml)	Absorbance	Mean absorbance	SD	RSD
10	0.051 0.053 0.053	0.053	0.0005	0.94
20	0.101 0.103 0.103	0.102	0.001	1.09
40	0.201 0.198 0.199	0.199	0.0015	0.75
60	0.306 0.303 0.308	0.305	0.0015	0.48
80	0.410 0.413 0.415	0.412	0.0055	1.33
100	0.510 0.515 0.513	0.512	0.005	1.00

Table 7: Different validation parameters of the calibration curve of Picrirrhiza kurroa extract

Parameter	Result
Linearity correlation co-efficient	0.999
Y - intercept	0.005
Slope	0.005
Range	10 – 180 µg/ml
LOD	0.062 µg/ml
LOQ	0.190 µg/ml

CONCLUSION

From the above studies, it can be concluded that the developed method of estimation of Picrorrhiza kurroa extract using UV Spectrophotometric technique can be used for direct and rapid measurement of the extract. This technique can be used for the estimation of Picrorrhiza kurroa extract in different formulations and can be highly helpful in formulation development, particularly in the dissolution studies.

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