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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE QUICK ESTIMATION OF *PIPER BETLE* LEAF (PBL) EXTRACT

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ABSTRACT

Piper betle leaves Linn. (Family: Piperaceae) have been used in Chinese and Indian folk medicine for centuries. *Piper betle* leaf extract obtained from simple maceration process. Calibration curve of leaf extract were prepared in phosphate buffer of pH 7.2 on three consecutive days at λ_{max} 280 nm. The absorbance values (mean of three determinations) with their standard deviations at different concentration in the range of 20-100 µg/ml. Extract was found to obey Beer-Lambert's law in the concentration range of 20-100 µg/ml with regression coefficient (r^2) values 0.9998. The regression equations were calculated as y = 5.2766 + 324.9606X for phosphate buffer of pH 7.2. The developed calibration curve was validated for intra-day and inter-day variations as per ICH Q2A guideline and was found to be a stable method.

Keywords: *Piper betle* Leaf Extract, Maceration process, Validation.

INTRODUCTION

The *Piper betle* plant is widely growing in the tropical humid climate of South East Asia, and its leaves, with a strong pungent and aromatic flavour, are widely consumed as a mouth freshener [1]. The leaves are credited with wound healing, digestive, and pancreatic lipase stimulant activities in the traditional medicine [2]. The deep green heart shaped leaves of betel vine are popularly known as *Paan* in India [3]. The scientific name of betel vine is *Piper betle* Linn. family Piperaceae [4].

There are no reported UV-visible methods for quick 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 calibration curve was developed using phosphate buffer of pH 7.2. The assay validation of calibration curve was carried out as per USP guidelines in category I and as per ICH Q2A guidelines. In validation procedure, calibration curve prepared in phosphate buffer of pH 7.2, was run in triplicate for 3 days to determine intra and inter day variations [5].

MATERIALS & METHODS

Materials

The PBL leaves were purchased from the local market of Varanasi, UP, India. All the other chemicals and reagents used in this study were of AR grade and were purchased from Ranbaxy Fine Chemicals, New Delhi.

Methods

Collection and Authentication of *Piper betle* leaves (PBL)

The PBL leaves were purchased from the local market of Varanasi, UP, India and authenticated from National Bureau of Plant Genetic Resources (ICAR), New Delhi. The voucher specimens are preserved in the department (NHCP/NBPGR/2009-6/372).

The collected material was cleaned and dried under shade (at ambient temperature), and then in oven at 20-40ºC. The dried leaves were weighed (2.5 kg) and stored in desiccator.

Extraction of plant material

The extraction was done by Simple Maceration Process [6]. The plant material (725 g) were mixed in double distilled water (9250 mL) and 3% chloroform, placed for 7 days under room temperature with occasional shaking. The mixture was filtered with muslin cloth, simple filter paper and then finally with Whatmann filter paper to obtain clear liquid extracts. The clear liquid extract was lyophilized at −80ºC to obtain dark brown colored crude extract, which was stored in desiccator until 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 preparation of calibration curve [7].

Scanning for λ_{max}

One hundred milligrams of crude extract was dissolved in little volume of phosphate buffer of pH 7.2 and finally diluted to 100 mL in volumetric flask to get a concentration of 1000 µg/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 200-400 nm using UV-spectrophotometer.

Preparation of calibration curve

Aliquots of the stock solution of PBL extract (1000 µg/mL) were pipetted out into a series of 10 mL volumetric flasks and diluted with phosphate buffer of pH 7.2 to get a final concentration of 20-100 µg/mL. The absorbance of the resultant solutions was measured at 280 nm. Freshly prepared solutions were made for the calibration curve on three consecutive days.

Validation of calibration curve

Assay validation of calibration curve was carried out as per USP guidelines in category I and as per ICH Q2A guidelines. In validation procedure, calibration curve prepared in phosphate buffer of pH 7.2, was run in triplicate for 3 days to determine intra and inter day variations [5].

RESULTS AND DISCUSSION

The PBL Extract was soluble in water, phosphate buffer of pH 7.2 and Dimethylsulphoxide (DMSO) and was insoluble in acetone, toluene, propane-2-ol, chloroform, methanol and ethanol (Table 1).

The λ_{max} of drug in phosphate buffer pH 7.2 was determined using UV-Spectrophotometer. The λ_{max} was determined by scanning 100 µg/mL solution of drug in the test medium in the range of 200-400 nm. The λ_{max} was found to be 280 nm and the absorbance was found to be 0.318.

Calibration curve of drug were prepared in phosphate buffer of pH 7.2 on three consecutive days at λ_{max} 280 nm. The absorbance values

(mean of three determinations) with their standard deviations at different concentration in the range of 20-100 µg/mL are given in Table 2. The calibration curve is given in Figure 1. The extract was found to obey Beer-Lambert's law in the concentration range of 20-100 µg/mL with regression coefficient (r^2) values 0.9998. The regression equations were calculated as y = 5.2766 + 324.9606X 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 and the percentage recovery was found to be in the range of 100.49-100.89, which was within the recommended tolerance of 80–115% [8]. The results are shown in Table 3.

The precision of an 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 0.61-1.9, 0.94 - 4.86 and 0.97- 5.28 for first, second and third days, respectively, in the intraday study and was found to be in the range of 0.98-7.75 in the inter day assay. The results are given in Tables 4 to 7. 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 15% RSD [9].

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 buffers 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 the stated experimental conditions. These two parameters are required for assay validation as per ICH Q2A 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) [10]. LOD and LOQ of calibration curve of drug prepared in phosphate buffer of pH 7.2. The results are given in Table 8.

Table 1: Solubility profile of the pbl extract

Solvent	Solubility behaviour
Water	Soluble
Phosphate buffer, pH 7.2	Soluble
DMSO	Soluble
Acetone	Insoluble
Toluene	Insoluble
Propane-2-ol	Insoluble
Chloroform	Insoluble
Methanol	Insoluble
Ethanol	Insoluble

Table 2: Calibration curve data of pbl extract in phosphate buffer of ph 7.2

Concentration (µg/ml)	Absorbance
20	0.051±0.0005
40	0.100±0.0038
60	0.169±0.0131
80	0.228±0.0118
100	0.294±0.0153

Table 3: Recovery results of drug for determination of accuracy

Labeled amount (µg)	Amount added (µg)	Amount recovered (µg)	Percentage recovery
25	25	50.44	100.89
75	25	100.49	100.49
125	25	150.85	100.57

Table 4: Results of intraday precision studies for day one

Conc (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.051	0.051	1.90
	0.051		
	0.052		
40	0.098	0.098	0.61
	0.097		
	0.098		
60	0.163	0.162	0.92
	0.161		
	0.164		
80	0.219	0.218	1.30
	0.215		
	0.221		
100	0.275	0.276	0.72
	0.279		
	0.276		

Table 5: Results of intraday precision studies for day two

Conc (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.053	0.053	0.94
	0.052		
	0.053		
40	0.097	0.098	1.02
	0.099		
	0.098		
60	0.197	0.187	4.86
	0.184		
	0.179		
80	0.243	0.238	1.97
	0.235		
	0.235		
100	0.303	0.299	1.34
	0.295		
	0.300		

Table 6: Results of intraday precision studies for day three

Conc (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.051	0.052	1.92
	0.051		
	0.053		
40	0.101	0.103	0.97
	0.104		
	0.103		
60	0.163	0.162	0.62
	0.162		
	0.161		
80	0.225	0.237	5.06
	0.236		
	0.250		
100	0.285	0.303	5.28
	0.309		
	0.316		

Table 7: Results of inter day precision studies of the calibration curves of PBL extract

Conc (µg/mL)	Absorbance	Mean absorbance	RSD (%)
20	0.051	0.051	0.98
	0.052		
	0.051		
40	0.097	0.100	3.80
	0.099		
	0.104		
60	0.161	0.169	7.75
	0.184		
	0.162		
80	0.215	0.228	5.10
	0.235		
	0.236		
100	0.279	0.294	5.20
	0.295		
	0.309		

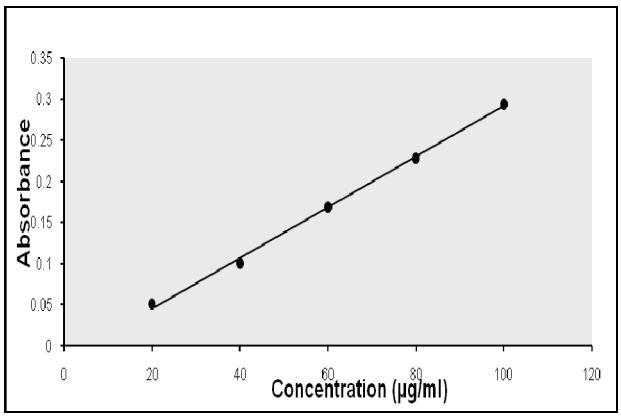

Fig. 1: Calibration curve of pbl extract

Table 8: Different validation parameters of the calibration curve of pbl extract

Parameters	Results
Linearity correlation coefficient	0.9998
y- intercept	5.2766
Slope	324.9606
Range	20-100 µg/mL
LOD	0.01556 µg/mL
LOQ	0.04714 µg/mL

CONCLUSION

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

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