Quantitative analysis of piperine in ayurvedic formulation by UV Spectrophotometry

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Abstract

A simple and reproducible UV- spectrophotometric method for the quantitative determination of piperine in Sitopaladi churna (STPLC) were developed and validated in the present work. The parameters linearity, precision , accuracy, and standard error were studies according to indian herbal pharmacopiea.

In this present study a new, simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of piperine in market and laboratory herbal formulation of Sitopaladi churna. which were procured and purchased respectively from the local market and they were evaluated as per Indian herbal Pharmacopoeia and WHO guidelines. The concentration of piperine present in raw material of PSC was found to be 1.45±0.014 w/w in piper longum fruits. Piperine has the maximum wavelength at 342.5 nm and hence the UV spectrophotometric method was performed at 342.5 nm. The samples were prepared in methanol and methos obeys Beers law in concentration ranges employed for evaluation. The content of piperine in ayurvedic formulation was determined. The result of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of Piperine in crude drug and its herbal formulation.

Key words: finger printing, piperine, Sitopaladichurna, UV-Spectrophotometer.

Introduction

The formulation of Sitopaladi churna (STPLC) is well known ayurvedic formulation is official in Ayurvedic Formulary of India, traditionally used for asthma, cough and cold, Tuberculosis, chest pain, epistaxis, chronic rhinitis / sinusitis, coryza and other respiratory disorders[3].Through it is very popular medicine, no establishment of quality control for this drug studies have been performed yet. This paper reports on instrumental methods for ensuring the Identity, Potency, Purity, Safety and efficacy of the Sitopaladi churna. This paper includes estimation of piperine for different samples of Sitopaladi churna (STPLC) and comparison studies between marketed formulations as STPLC-A, STPLC-B and STPLC-C and lab formulations as STPLC-I, STPLC-II, STPLC-III by UV spectroscopic methods.

Spectroscopic studies were carried out to develop the spectrum of the formulation and validated by overlain and linearity study (Beer's law).The results of all batches were found in close proximity with each other. The methods used for determination of Sitopaladi churna found to be precise, reproducible and can be considered for routine quality control and finger printing of the formulation.The present study is an attempt to develop the fingerprinting method for Sitopaladi churna (STPLC) by spectrophotometric determination using Piperine as a standard, which is an importanat and major content in formulation. The developed spectroscopic fingerprints can be used as a standard and piperine can be used as a possible marker compound for fingerprinting of STPLC.

1.Material and Methods:

Procurement of crude drug

Sitopaladi churna (STPLC) consist of 5 ingredients viz., *Sitopala ,Bambusa bambos, Piper longum, Cinnamommum zeylanicum, Elettaria cardamomum.*

All these 5 ingredients were procured from local market and identified morphologically[1], microscopically and compared with standard Pharmacopoeial monograph[4,5,6,7]. Samples of crude drugs were also authenticated by Department of Botany, Dr. H.S.Gour Vishwavidyalaya ,Sagar (M.P.)

Preparation of the formulation STPLC

Three marketed formulation of Sitopaladi churna from different manufactures (designated as STPLC-A, STPLC-B and STPLC-C) and three laboratory batches of Sitopaladi churna were prepared in local laboratory and were named as STPLC-I ,STPLC-II, STPLC-III. were procured for the present study[2].

2. Chemicals

All the chemicals and solvents were used of A.R. grade, standard piperine(98%) was procured from Lancaster(England).

Preparation of piperine extract of Sitopaladi churna

Reflux the powderd Sitopaladi churna(1gm) with 60 ml ethanol for 1 hour. Filter the extract and reflux the marc left with 40 ml of methanol for another 1 hour.filter and combine the filtrate. Concentrate the methanol extract under vaccum till the semisolid mass is obtained.dissolve the residue in 75 ml methanol and filter through sintered glass funnel (G-2) by vaccum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100ml volumetric flask and volume was made with methanol. The same procedure was performed for each batch of Sitopaladi churna and separately powderd fruits of piper longum (Pippli) and solution (100ml)of their piperine extract were prepared[8].

Preparation of standard solution of piperine

An accurately weighed piperine(100mg)was dissolved in methanol and volume was made up to 100ml with methanol in volumetric flask. Two ml of this solution was diluted with methanol up to 100ml in volumetric flask to give 20µg/ml piperine solution.

Experimental

Calibration curve from standard solution of piperine was prepared and with the help of this curve the content of piperine from Sitopaladi churna was estimated. The method was validated for precision and accuracy.

Calibration curve of piperine in STPLC

A series of calibrated 10ml volumetric flask were taken and appropriate aliquots of the working standard solution of piperine were withdrawn and diluted up to 10ml with methanol.the absorbance was measured at absorption maxima 342.5 nm, against the reagent blank prepared in similar manner without the piperine. The absorption maxima and Beer's law limit were recorded and data that prove the linerity and obey Bee'r law limit were noted.

The linear correlation between these concentrations(x-axis) and absorbance(y-axis) were graphically presented and slope(b), intercept(a), and correlation confficiant (r^2) were calculated for the linear equation (Y=bx+a) by regression using the methanol of the least square, Table1 figure1.

Validation parameters of piperine in STPLC (Mean% ± SD,n=3): Table-1

Sno.	parameters	value
01	Absorption maxima	342.5nm
02	Beer's law limit	10-50ug/ml
03	Regression equation(y=bx+a)	0.013x+0
04	Intercept(a)	0
05	Slope(b)	0.013
06	Correlation coefficients(r2)	0.9961
07	Precision (n=6, % RSD)	0.978
08	Accuracy(%)	99.03

Calibration curve of piperine: (figure1).

s.no.	Concentration (µgm/ml)	absorbance
01	10	0.15
02	20	0.269
03	30	0.395
04	40	0.518
05	50	0.641

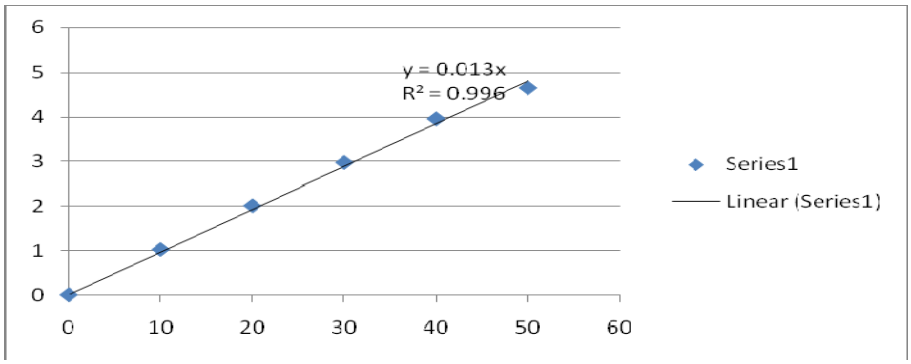

Figure-1: Calibration curve of piperine in STPLC

Estimation of piperine in STPLC

The appropriate aliquots from piperine extract of each batch of Sitopaladi churna and separately powderd fruits of piper longum (Pippli) were withdrawn in 10ml volumetric flask separately absorbance for aliquots of each was noted at 342.5 nm. the corresponding concentration of piperine against respective absorbance value was determines using the piperine calibration curve. The statistical analysis for checking uniformity in batches is also performed below(Table-2).

s.no.	name		Piperine content %w/w
01	piper longum(pippli)		1.45±0.34
02	SITOPALADI CHURNA	STPLC-I	0.31±0.008
03		STPLC-II	0.34±0.002
04		STPLC-III	0.32±0.003
05		STPLC-A	0.70±0.002
06		STPLC-B	0.72±0.005
07		STPLC-C	0.75±0.006

Precision and accuracy

The methanol was validated for precision and accuracy by performing the recovery studies at two levels by adding known amount of piperine extract of Sitopaladi churna, of which the piperine content have been estimated previously. The data were obtained and recovery was calcuted.(Table-3).

Data of recovery study (Mean% ± SD,n=3): .(Table-3).

S.no.	Amount of piperine(µgm/ml)			RSD%	SE	Recovery%
	In sample	added	estimated			
01	100	50	149.62±0.86	0.57	0.32	99.74
02	100	100	199.26±0.42	0.21	0.12	99.63
03	100	150	249.30±0.12	0.04	0.023	99.72
mean				0.27	0.15	99.69

Mean ± SD of six determinations, RSD= Relative standard deviation , SE=Standard error

Results and Discussion

Piperine obeys Beer Lambert'law in concentration range 10-50µg/ml at the λ_{max} 342.5 nm. The correlation coefficiant (r^2) was calculated where the (r^2) value 0.9961 indicates the good linearity between the concentration and absorbance.

The estimation of piperine in Sitopaladi churna (laboratory batch STPLC-I, STPLC-II and STPLC-III and marketed formulations STPLC-A, STPLC-B, and STPLC-C) and powderd fruits of piper longum was carried out separately. The concentration of piperine present in raw material was found to be 1.45±0.341 w/w in piper longum fruit. The concentration of piperine in different batches of STPLC(laboratory batches STPLC-I, STPLC-II and STPLC-III)was found to be 0.31±0.008%, 0.34±0.002 %,0.32±0.003 % and in marketed formulations STPLC-A, STPLC-B, and STPLC-C was found to be 0.70±0.002 %, 0.72±0.005 %, 0.75±0.006 % respectively(In Table-2).

In order to optain precision and accuracy the recovery study were performed at three levels by adding known amount of piperine with preanalysed sample of piperine in Sitopaladi churna (STPLC). The experment was repeated Six Times at both level(Table-3) and result shows 99.74%, 99.63%,and 99.72% recovery of piperine at all the level with mean value 99.69%, which prove reproductibility of the result. This shows significant precision of methods at 95% confidence level. The % relative standard deviation(%RSD) value was found to be 0.57, 0.21,and 0.04, with mean 0.27 at all the level while the standard error was 0.32, 0.12 and 0.023 with Mean 0.15 respectively. From the data it is obvious that the present method of UV-Spectrophotometric fingerprinting determination of Piperine is simple, precise, accurate, and suitable for routine analysis of Piperine in Sitopaladi churna (STPLC).

Conclusion

Development and validation of spectrophotometric method for the estimation of piperine in Sitopaladi churna could be used as a valuable analytical tool in routine analysis, to check the batch to batch variations After the drug is approved , pharmaceutical validation and development of finger printing and are necessary to ensure that the drug product will meet/set pharmaceutical standards for identity, strength, quality,purity, stability, evaluation safety and efficacy. In general, pharmaceutical development of finger printing provide a certain assurance of batch uniformity and integrity of the product manufactured. Estimation of piperine can be used as one of the appropriate analytical markers for the finger printing.

Acknowledgement

The authors are grateful to Principal, BITS-Pharmacy college , Bhopal for their unforgetable support**.**

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