



## Development of finger printing methods of an ayurvedic formulation bhaskar lavan churna: A spectrophotometric approach

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### ABSTRACT

Bhaskar lavan churna is a well known ayurvedic formulation containing Piper species (Fruits of piper longum, Piper nigrum and roots of piper longum) as main ingredients. The aim of present study is to develop finger printing methods for Bhaskar Lavan churna. Three batches of bhaskar lavan churna(BLC) were prepared according to the method given in the ayurvedic formulary and another three different formulations were procured from the market. In present study a spectrophotometric method was developed for the estimation of Piperine in laboratory and marketed preparations. The concentration of piperine present in raw material of BLC was found to be  $3.49\% \pm 0.021$  w/w in piper nigrum fruits,  $1.52\% \pm 0.18$  w/w in Piper longum fruits and  $1.12 \pm 0.20$  w/w in piper longum roots. The content of Piperine in laboratory preparation was found to be  $(0.18 \pm 0.124\%)$  and in different marketed preparations of bhaskar lavan churna was for BLC-A( $0.08 \pm 0.212\%$ ), BLC-B( $0.092 \pm 0.36\%$ ), BLC-C( $0.068 \pm 0.42\%$ ) w/w respectively. In order to obtain Precision and Accuracy the Recovery study was performed and result obtained with mean value  $99.49\% \pm 0.19$ , which prove reproducibility of the result. This shows significant Precision of methods at 95% confidence level. The mean of %RSD value was found to be 0.327 with the mean standard error 0.240. A result of statistical analysis show present spectrophotometric method for determination of piperine is simple, precise, accurate and suitable for routine analysis of piperine in bhaskar lavan churna. The developed fingerprints can be used as a standard and piperine can be used as a possible marker compound for fingerprinting of the formulation.

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### KEYWORDS

Finger printing;  
Piperine;  
Bhaskar lavan churna;  
Spectrophotometric method;  
Ayurvedic formulation.

### INTRODUCTION

India has a vast heritage of traditional system of medicine(Ayurveda, siddha and unani), owing to lack of precise quality control measures, the benefits from these systems remains largely underutilized<sup>[1]</sup>. Keeping those facts in mind from the last two decades, efforts

have been made to develop parameters of quality control for traditional formulations (Ayurvedic formulations) by physiochemical parameters, spectroscopic and finger printing profile. The bhaskar lavan churna polyherbomineral ayurvedic formulation described in ayurvedic formulary of India, traditionally used for digestive impairment, rheumatism, dyspepsia, malabsorp-

tion syndrome, angina pectoris, disease of skin, splenic disorder, oedema, asthma and constipation<sup>[2,4]</sup>. Among all the ayurvedic formulation for the Gastro intestinal disturbance bhaskar lavan churna is the most preferred by the ayurvedic practitioners of India.

Ayurvedic formulary of India has given the specification for the composition of bhaskar lavan churna, it should contain piper species as a major ingredient(12% of total) apart from different herbs and salts.

The World health organization(WHO) assembly in its resolution WHA 31.33(1978), WHA 40.33(1987) and WHA 42.43(1989) has emphasized the need to ensure the quality of the medicinal plants products by using modern controlled techniques and applying suitable standards<sup>[3]</sup>. The present study is an attempt to develop the fingerprint method for bhaskar lavan churna by spectrophotometric determination using Piperine as a standard, which is as an important 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 BLC.

## Materials and methods

### Procurement of crude drug

Crude drugs were procured from local market and identification was conformed by macroscopic and microscopic characters<sup>[6,7,8]</sup>.

### Preparation of the formulation

Three batches were prepared in laboratory according to reported method of ayurvedic formulary of India<sup>[2]</sup>. The available commercially brands BLC-A, BLC-B and BLC-C of bhaskar lavan churna were procured from local pharmacy.

### 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 bhaskar lavan churna

Reflux the powdered Bhaskar lavan churna (1gm) with 60ml ethanol for 1hour. Filter the extract and re reflux the marc left with 40ml of ethanol for another 1hours. Filter and combine the filtrate. Concentrate the ethanol extract under vacuum till the semisolid mass is

obtained. Dissolve the residue in 75ml ethanol and filter through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000rpm for 20minutes, the supernatant was collected in 100ml volumetric flask and volume was made with ethanol<sup>[9,10]</sup>.

The same procedure was performed for each batch of Bhaskar lavan churna and separately powdered fruits of Piper longum(Pippali), Piper nigrum(Marica), roots of Piper longum(Pippali) and solution(100ml) of their piperine extract were prepared.

### Preparation of standard solution of piperine

An accurately weighed piperine(100mg) was dissolved in ethanol and volume was made up to 100ml with ethanol in volumetric flask. Two ml of this solution was diluted with ethanol up to 100ml in volumetric flask to give 20µg/ml piperine solution<sup>[9,10]</sup>.

## EXPERIMENTAL

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

### Calibration curve of piperine

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 ethanol. The absorbance was measured at absorption maxima 342.5nm, 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 linearity and obey Beer's law limit were noted.

The linear correlation between these concentrations (X-axis) and absorbance(Y-axis) were graphically presented and the slope(b), intercept(a), and correlation coefficient( $r^2$ ) were calculated for the linear equation( $Y = bx + a$ ) by regression analysis using the method of the least square, TABLE 1 and figure 1.

### Estimation of piperine

The appropriate aliquots from piperine extract of each batch of bhaskar lavan churna and separately fruits of Piper longum (Pippali) Piper nigrum(Marica) and

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**TABLE 1 : Optical characteristics, statistical regression data and validation parameter of piperine**

S. No.	Parameter	Value
1	Absorption maxima	342.5nm
2	Beer's law limit	2-20 $\mu$ g/ml
3	Regression equation (y= bx+a)	y=0.0909 $\times$ 0.0558
4	Intercept (a)	0.909
5	Slope (b)	0.0558
6	Correlation coefficients (r <sup>2</sup> )	r <sup>2</sup> = 0.9989
7	Precision (n=6, %RSD)	0.327
8	Accuracy (%)	99.48

**TABLE 2 : Estimation of piperine content**

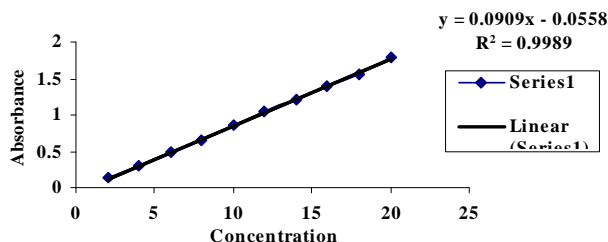
S. No	Name	Piperine content %w/w	Confidence level (95%)
1	Piper nigrum (Marica)	3.49 $\pm$ 0.21	$\pm$ 0.180
2	Piper longum (Pippali) fr.	1.52 $\pm$ 0.16	$\pm$ 0.282
3	Piper longum (Pippali) rt.	1.12 $\pm$ 0.20	$\pm$ 0.192
4	Bhaskar BLC L	0.18 $\pm$ 0.124	$\pm$ 0. 212
5	lavan BLC A	0.08 $\pm$ 0.410	$\pm$ 0. 240
6	churna BLC B	0.092 $\pm$ 0.36	$\pm$ 0. 278
7	BLC C	0.068 $\pm$ 0.042	$\pm$ 0. 342

Mean $\pm$ SD of six determinations, BLC L: Bhaskar lavan churna laboratory batch, BLC A: marketed formulation I, BLC B: marketed formulation II, BLC C : marketed formulation III, Fr.=Fruits, Rt.=Roots

**TABLE 3 : Data of recovery study**

S. No	Amount of Piperine ( $\mu$ g/ml)			RSD%	SE	Recovery%
	In sample	Added	Estimated			
1	100	50	149.4 $\pm$ 0.61	0.477	0.289	99.26 $\pm$ 0.12
2	100	100	199.2 $\pm$ 0.78	0.391	0.0319	99.6 $\pm$ 0.12
3	100	150	249.03 $\pm$ 0.28	0.112	0.114	99.6 $\pm$ 0.34
<b>Mean</b>				0.327	0.240	99.48

Mean $\pm$ SD of six determinations, RSD=Relative standard deviation, SE=Standard error



**Figure 1 : Calibration curve of piperine**

roots of Piper longum(Pippali) were withdrawn in 10ml volumetric flask separately absorbance for aliquots of each was noted at 342.5nm. 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(TABLE 2)

Precision and accuracy: The method was validated for precision and accuracy, by performing the recovery studies at two levels by adding known amount of piperine extract of bhaskar lavan churna, of which the piperine content have been estimated previously. The data were obtained and recovery was calculated (TABLE 3).

## RESULTS AND DISCUSSION

Piperine obeys Beer Lambert's law in concentration range 2-20 $\mu$ g/ml at  $\lambda_{max}$  342.5nm. The correlation coefficient (r<sup>2</sup>) was calculated where the r<sup>2</sup> value 0.9989 indicates the good linearity between the concentration and absorbance.

The estimation of piperine content of bhaskar lavan churna(three identical laboratory and three marketed samples) and powdered fruits and roots of piper longum (Pippali) and fruits of piper nigrum(Marica) was carried out separately. The concentration of Piperine present in raw material was found to be 3.49 $\pm$ 0.021w/w in piper nigrum fruits, 1.52 $\pm$ 0.18 w/w in Piper longum fruits and 1.12 $\pm$ 0.20 w/w in piper longum roots. Content of piperine in different batches of BLC(marked and laboratories batches) was found to be 0.18 $\pm$ 0.124%, 0.08  $\pm$ 0.212%, 0.092 $\pm$ 0.36 % and 0.068 $\pm$ 0.42 % w/w respectively for BLC-L, BLC A, BLC B and for BLC C(TABLE 2).

In order to obtain precision and accuracy the recovery study were performed at three levels by adding known amount of piperine with preanalysed sample of piperine in bhaskar lavan churna. The experiment was repeated six times at both level (TABLE 3) and result shows 99.26 $\pm$ 0.26, 99.6 $\pm$ 0.12 and 99.6 $\pm$ 0.34 recovery of Piperine at all the level with mean value 99.48 $\pm$ 0.19, which prove reproducibility of the result. This shows significant precision of methods at 95% confidence level. The % relative standard deviation (% RSD) value was found to be 0.477, 0.391 and 0.112 with mean 0.327 at all the level while the standard error was 0.289, 0.319 and 0.114 with mean 0.240 respectively. From the data it is obvious that the present method of Spectrophotometric determination of Piperine is simple, precise, accurate and suitable for routine analysis of piperine in bhaskar lavan churna.

## CONCLUSION

The developed spectroscopic method for estimation of Piperine from *Bhaskar Lavan churna* could be used as a valuable analytical tool in the routine analysis, to check the batch to batch variation. Estimation of Piperine can be used as one of the appropriate analytical markers for the finger printing.

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