

Development of UV spectrophotometric fingerprint method for evaluation of Sitopaladi churna

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ABSTRACT

Sitopaladi churna is a most trusted and sold Ayurvedic formulation among the Ayurvedic medicines. Sitopaladi churna contains sugar, *Bombusa bombos* (Banslochan), *Piper longum* (Pipali), *Elattaria cardamomum* (Elaichi), *Cinnomomum zeylanicum* (Tvak). Piperine, 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine is the alkaloid [1] a biomarker constituent of *Piper longum* (fruit) responsible for the pungency of piper longum(fruit) .It has also been used in some forms of traditional medicine and as an insecticide. Piperine extract was prepared from the fruits of *Piper longum* and two lab formulations and three marketed formulations. Piperine was used as a reference standard for analysis of *P. longum* in lab formulations and marketed formulations. Content of piperine in different batches of Sitopaladi churna (laboratories and marketed batches) was found to be $0.456 \pm 0.015\%$, $0.432 \pm 0.022\%$ and $0.188 \pm 0.002\%$, $0.172 \pm 0.001\%$ and $0.165 \pm 0.001\%$ w/w respectively for SL-I, SL-II and SCM- A, SCM-B and SCM-C. A rapid accurate and precise method was developed in the laboratory. The proposed method can be used for uv-spectrophotometric fingerprint analysis of piperine in Sitopaladi churna.

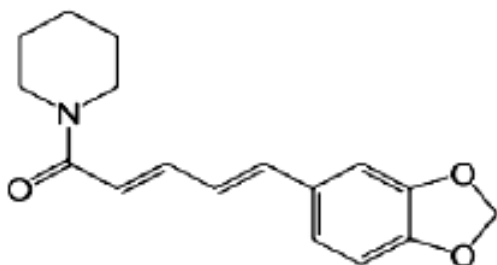
Key words: Piperine, fingerprint, UV spectrophotometer, Sitopaladi churna.

INTRODUCTION

Herbal fingerprinting not only gives the idea for authentication of drugs and its constituents but also establish parameters for quality of herbal formulations by different sophisticated instruments³. Sitopaladi churna is used for different disorders like intercostal neuralgia and pleurodynia, asthma due to disorder of bile, numbness of tongue, digestive impairment, burning sensation in palm and soles².

Piperine is one of the major constituent of Sitopaladi churna. Molecular formula $C_{17}H_{19}NO_3$, density 1.193 g/cm^3 , melting point 130°C , in animal studies, piperine also inhibited other enzymes important in drug metabolism⁴, recently researchers discovered that Piperine can stimulate pigmentation in the skin⁵.

Sitopaladi churna is mentioned in sarangdhara samhita madhymakhanda adhyay 6,134-135 1/2(old Ayurvedic classical text). The two lab formulations were prepared as per Ayurvedic formulary of India and the three marketed formulations were purchased for the estimation of piperine. One Separately powdered Drug of piper longum(fruit)'s piperine extract was also taken in this study .since the other ingredient of the formulations does not contain piperine so they were not included in the present study. The study was taken on the basis of method development which is a fingerprint of Sitopaladi churna by means of



piperine content Spectroscopically. The reported method is accurate precise useful for routine Uv-spectrophotometric fingerprint analysis of piperine in Sitopaladi churna Using piperine as a biomarker.

MATERIALS AND METHODS

Procurement of crude drug

Crude drugs were procured from local market and identification was conformed by macroscopic and microscopic Characters.

Preparation of the formulation

Two batches were prepared in laboratory according to reported method of Ayurvedic formulary of India .The available commercial brands SCM-A, SCM-B and SCM-C of Sitopaladi 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 sitopaladi churna^{6,7}

Sitopaladi churna (1gm) powder was refluxed with 60ml ethanol for 1hour. Filtered the extract and re reflux the marc left with 40ml of ethanol for another 1hours. Filter and combine the filtrate. The ethanol extract was Concentrated under vacuum till the semisolid mass is obtained. Dissolved 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 ethanol6. The same procedure was performed for each batch of

Sitopaladi churna and separately powdered fruits of a Piper longum (Pippali) and solution (100ml) of their piperine extract were prepared.

Preparation of standard solution of piperine

An accurately weighed piperine (10 mg) was dissolved in ethanol and volume was made up to 100ml with ethanol in volumetric flask. The final solution was 100 µg of piperine in per ml of 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 and piper longum (fruit) 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 342.2 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 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(a), Intercept(b), and correlation coefficient(r²) were calculated for the linear equation $y^* = b + ac$ by regression analysis using the method of the least square, result given in Table 1.

Estimation of piperine

The appropriate aliquots from piperine extract of each batch of sitopaladi churna and separately fruits of Piper longum (Pippali) and three marketed formulations were withdrawn in 10 ml volumetric flask separately from the extract and diluted with ethanol and the absorbance was noted at 342.2nm.

RESULTS AND DISCUSSION

The developed method was found to be reliable, accurate, and precise. The method involves absorbance measurement at 342.2 nm for Piperine corresponding to the absorption maxima of the herbal formulation Sitopaladi churna. The optical

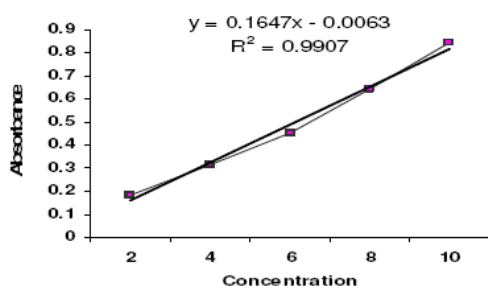


Fig. 1: Calibration curve of piperine

characteristic shows that Piperine obeys Beer Lambert's law in concentration range 2-10µg/ml at - λ max 342.2 nm. The correlation coefficient (r2), Regression equation, Precision and Accuracy were calculated for the spectrophotometric method and results are summarized in table 2.

churna (two laboratory and three marketed samples) and powder of piper longum(fruit) (Pippali) was carried out separately. The concentration of Piperine present in raw material was found to be 0.535 ± 0.016 w/w in Piper longum(fruit). ontent of piperine

The r2 value 0.9907 indicates the good linearity between the concentration and absorbance. The estimation of piperine content of Sitopaladi

Table 2: Optical and regression characteristics of Piperine

Parameters	Observations
Absorption maxima	342.2 nm
Beer's law limit (µg/ml)	2-10
Correlation coefficient (r2)	0.9907
Regression equation (y*)	Y = 0.1647-0.0063
Slope (a)	0.1647
Intercept (b)	0.0063

y* = b + ac, where "C" is concentration in µg/ml and y is absorbance unit.

Table 1: Calibration data of Piperine

S. No.	Conc. (µg/ml)	Absorbance
1	2	0.184
2	4	0.314
3	6	0.454
4	8	0.645
5	10	0.842

Table 3: Estimation of Piperine content (% (w/w))

S. No	Name	Piperine content % (w/w)	Standard error of mean (SEM)	Confidence level (95%)
1.	<i>Piper longum</i> (fruit)	0.535 ± 0.016	0.0065	± 0.5117
2.	SL-I	0.456 ± 0.015	0.0062	± 0.4099
3.	Sitopladi SL -II	0.432 ± 0.022	0.0090	± 0.4064
4.	Churna SCM-A	0.188 ± 0.002	0.0008	± 0.1837
5.	SCM-B	0.172 ± 0.001	0.0005	± 0.1724
6.	SCM-C	0.165 ± 0.001	0.0006	± 0.1617

SL=lab batch sitopaladi churna , SCM=marketed sitopaladi churna

Table 4: Results of recovery of Piperine in Sitopaladi churna

S. No	Formulation Name	Conc. of sample (µg/ml)	Amount of drug added			% Recovery				%C
			5	10	15	I	II	III	AVG	
1	Pipali	10	14.91	19.81	24.89	99.4	99.05	99.56	99.33	0.26
2	SL-I	10	14.78	19.65	24.79	98.53	98.25	99.16	98.64	0.47
3	SL-II	10	14.72	19.60	24.72	98.13	98.0	98.88	98.33	0.48
4	SCM-A	10	14.32	19.36	24.12	95.46	96.8	96.48	96.24	0.72
5	SCM-B	10	14.23	19.26	24.09	95.46	96.3	96.36	96.04	0.52
6	SCM-C	10	14.01	19.06	24.02	94.2	95.3	96.08	95.19	0.99

in different batches of Sitopaladi churna (laboratories and marketed batches) was found to be $0.456 \pm 0.015\%$, $0.432 \pm 0.022\%$ and $0.188 \pm 0.002\%$, $0.172 \pm 0.001\%$ and $0.165 \pm 0.001\%$ w/w respectively for SL-I, SL-II and SCM- A, SCM-B and SCM-C. Results are summarized in table 3.

In order to obtain precision and accuracy the recovery study were performed by adding known amount of piperine with pre-analyzed sample of piperine in Sitopaladi churnas. The result shows

99.33%, 98.64%, 98.33% and 96.24%, 96.04% and 95.19% recovery of Piperine in piper longum, SL-I, SL-II and SCM- A, SCM-B and SCM-C respectively which shows reproducibility of the result. Results are summarized in table-IV.

The proposed method was found to be accurate, simple and rapid for fingerprint analysis of Piperine in Ayurvedic formulation Sitopaladi churna and could assist for checking the batch to batch variations in herbal formulations.

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