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Research Article

UV SPECTROPHOTOMETRIC DETERMINATION OF PIPERINE IN NAVASAYA CHURNA: A POLYHERBAL FORMULATION

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ABSTRACT

Navayasa Churna is an important ayurvedic formulation, is mentioned in Bhaishajyaratnavali in Pandu roga chikitsa is combination of Nine i.e. Amlaki, Bibhitaka, Haritaki, Marica Pippali, Sunth, Chitraka, Musta, Vidanga and Lauha bhasma. The formulation is dispensed for the treatment of Anemia (pandu), Hepatoprotective and Liver disorders. The method for spectrophotometric determination of piperine from the fruits of *Piper longum*, *Piper nigrum* and Navayasa Churna has been developed at absorption maxima 342.7nm. The concentration of piperine present in raw material was found to be 2.981 ± 0.38 % (w/w) in marica and 0.981 ± 0.047 % (w/w) in pippali, respectively and in three identical laboratory batch of Navayasa churna name NY-I, NY-II, and NY-III, was 0.223 ± 0.34 , 0.219 ± 0.42 , 0.215 ± 0.43 % (w/w), respectively with mean value 0.219 ± 0.903 % (w/w). The piperine content of all the three batches is found to be in close proximities with each other.

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INTRODUCTION:

World health organization reported that 70% of the population in the developing countries relies on herbal or traditional medicines for their primary health care Ayurveda is our ancient system of medicine in India and various ayurvedic medicines are being used since vedic period. Most of the ayurvedic formulations are lacked in their defined quality control parameters and method of its evaluation¹. World health organization has emphasized the need to ensure the quality and safety of medicinal plant products by using modern analytical and controlled techniques and applying suitable standards².

Navayasa Churna is well known ayurvedic formulation, comprised of the nine important medicinal plants *Embelica officinalis*, *Terminalia bellerica*, *Terminalia chebula*, *Piper nigrum*, *Piper longum*, *Zingiber officinalis*, *Plumbago zylenica*, *Cyperus rotundus*, *Embelia ribes* and Lauha bhasma. Navayasa churna comprised of fruits of *Piper nigrum* and *Piper longum* which contains piperine. Navayasa churna is used in the treatment of Anemeia (Pandu), Jaundice (kamala), Heart diseases (hrdroga), Piles (arsa) and Liver diseases³. Navayasa churna plays an essential role in the treatment of a wide variety of conditions. The present study was an attempt to develop the fingerprint method for

Navayasa churna by UV spectrophotometric determination using piperine as a standard is an important and major content in formulation. The UV spectrophotometric analysis can be considered as one of the quality control methods for routine analysis.

EXPERIMENTAL:

The crude drugs were procured from local market of Indore, India and identified on the basis of morphological and microscopical characters and compared with standard Pharmacopoeial Monograph³. All the chemicals and solvents were used of AR Grade. Standard Piperine (98%) was procured from Sigma Aldrich.

Preparation of Navayasa churna

Navayasa churna, three batches name NY-I, NY-II, NY-III, were prepared in laboratory using method described in ayurvedic formulary. These three batches of Navayasa churna and powdered *Piper longum* and (Pippali), *Piper nigrum* (Marica), were estimated for their piperine contents against standard piperine solution on UV-Visible spectrophotometer (Shimadzu, UV-1700, Pharmaspec). As other ingredients not contain piperine are not included in present study.

Preparation of piperine extract of Navasaya churna

Reflux the powdered Navasaya churna (1 g) with 60 mL ethanol for 1 h. Filter the extract and reflux the marc left with 40 mL of ethanol for another 1 h. Filter and combine the filtrate. Concentrate the ethanol extract under vacuum till the semisolid mass is obtained. Dissolve the residue in 75 mL ethanol and filter through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 min, the supernatant was collected in 100 mL volumetric flask and volume was made with ethanol.

The same procedure was performed for each batch of Navasaya churna and separately powdered *Piper longum* (Pippali) and *Piper nigrum* (Marica) and solution (100 mL) of their piperine extract were prepared.

Preparation of standard solution of piperine

An accurately weighed piperine (100 mg) was dissolved in ethanol and volume was made up to 100 mL with ethanol in volumetric flask. 1 mL of this solution was diluted with ethanol up to 100 mL in volumetric flask to give 10 mg/mL piperine solution. Calibration curve from standard solution of piperine was prepared and with the help of this curve the piperine of Navasaya Churna was estimated. The method was validated for precision and accuracy.

Calibration curve of piperine

A series of calibrated 10 mL volumetric flask were taken and appropriate aliquots of the working standard solution of piperine were withdrawn and diluted up to 10 mL with ethanol. The absorbance was measured at absorption maxima 342.7 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 (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).

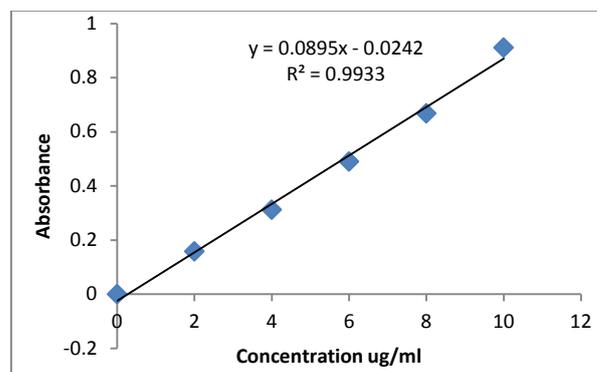


Figure 1: Calibration curve of piperine

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

S.N.	Parameter	Value
1	Absorption Maxima	342.7 nm
2	Beer's Law limit	2-10 ug /ml
3	Regression equation ($y = bx + a$)	$y = 0.0895x - 0.0242$
4	Intercept (a)	0.0895
5	Slope (b)	0.0242
6	Correlation coefficients (r^2)	$r^2 = 0.9933$
7	Precision (n=6, % RSD)	0.351
8	Accuracy (%)	99.2

Estimation of piperine

The appropriate aliquots from piperine extract of each batch of Navasaya churna and separately *Piper longum* (Pippali) and *Piper nigrum* (Marica) were withdrawn in 10 mL volumetric flask separately. The absorbance for aliquots of each was noted at 342.7 nm. The Corresponding concentration of piperine against respective absorbance value was determined using the piperine calibration curve. The statistical analysis for checking uniformity in batches is also performed (Table-2)

Table 2: Estimation of piperine content in Navasaya Churna

S.N.	Name	Piperine content %w/w	Confidence level (95%)
1	Piper longum	0.981 ± 0.047	± 0.494
2	Piper nigrum	2.89 ± 0.38	± 0.268
3	Navasaya Churna	NY-I	0.223 ± 0.34
		NY-II	0.219 ± 0.42
		NY-III	0.215 ± 0.43

Mean \pm SD of six determinations, NY-I: Navasaya Batch I, NY- II: Navasaya Churna Batch II, NY-III: Navasaya Churna Batch III.

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 Navasaya churna, of which the piperine content have been estimated previously. The data were obtained and recovery was calculated (Table-3).

Table 3: Compilation data of recovery study

S.no.	Amount of piperine ($\mu\text{g/ml}$)			RSD%	SE	Recovery%
	Sample	Added	Estimated			
1	100	50	149.03 \pm 0.62	0.409	0.25	99.24 \pm 0.25
2	100	100	198.22 \pm 0.57	0.292	0.237	99.13 \pm 0.23
Mean				0.551	0.248	99.18

Mean \pm SD of six determinations, RSD =Relative Standard Deviation, SE = Standard Error

RESULTS AND DISCUSSION:

Piperine obeys Beer Lambert's law in concentration range 2-10 $\mu\text{g/ml}$ at λ_{max} 342.7 nm. The correlation coefficient (r^2) was calculated where the r^2 value 0.9933 indicates the good linearity between the concentration and absorbance. The estimation of piperine content of Navasaya churna (three identical laboratory batch) and powdered *Piper longum* (Pippali) and *Piper nigrum* (Marica) was carried out separately. The concentration of piperine present in raw material was found to be 2.89 \pm 0.38 % (w/w) in marica and 0.981 \pm 0.047 % (w/w) in pippali, respectively and in three identical laboratory batches of Navasaya Churna name NY-I, NY -II, NY -III, was 0.223 \pm 0.34, 0.219 \pm 0.42, 0.215 \pm 0.43 % (w/w) (Table-2) respectively with mean value 0.219 \pm 0.903 % (w/w).

In order to obtain precision and accuracy, the recovery study was performed at two levels by adding known

amount of piperine with pre analyzed sample of piperine in Navasaya Churna. The experiment was repeated six times at both level (Table-3) and result shows 99.24 \pm 0.25 and 99.13 \pm 0.23 % recovery of piperine at both the level with mean value 99.18 \pm 0.25 %, 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 0.409 and 0.292 with mean 0.551 at both the level while the standard error was 0.25 and 0.237 with mean 0.248, 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 Navasaya churna.

As Navasaya churna is a good source of piperine, these findings can be taken as one of the parameter, along with other parameters, for quality control of Navasaya Churna.

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