Asian Journal of Chemistry Vol. 19, No. 7 (2007), 5331-5335

Spectrophotometric Determination of Piperine in Trikatu Churna: An Ayurvedic Formulation

VISHAL JAIN, S. SARAF and SWARNLATA SARAF*
Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur-492 010, India
E-mail: shailendrasaraf@rediffmail.com

Trikatu churna is an important ayurvedic formulation, is official in ayurvedic formulary of India is combination of three reputed herbs, comprised of the fruits *Piper longum* (Pippali), *Piper nigrum* (Marica) and rhizomes of *Zingiber officianalis* (Saunth). The formulation is dispensed for the disorder of respiratory tract and digestive system. The method for spectrophotometric determination of piperine from the fruits of pippali, marica and Trikatu churna have been developed at absorption maxima 342.5 nm. The concentration of piperine present in raw material was found to be 3.6 ± 0.31 % (w/w) in marica and 1.4 ± 0.27 % (w/w) in pippali, respectively and in three identical laboratory batch of Trikatu churna name TK-I, TK-II, TK-III, was 1.66 ± 0.39, 1.71 ± 0.42, 1.69 ± 0.43 % (w/w) (Table-2), respectively with mean value 1.69 ± 0.41 % (w/w). The piperine content of all the three batches is found to be in close proximities with each other.

Key Words: Ayurvedic formulation, Spectrophotometric estimation, Piperine, Trikatu churna.

INTRODUCTION

Most of the ayurvedic formulation are lacked in their defined quality control parameters and method of its evaluation[1]. World health organization has emphasized the need to ensure the quality of medicinal plant products by using modern controlled technique and applying suitable standards[2]. The present paper is an effort to develop the quality control parameter of Trikatu churna by spectrophotometric determination using piperine as a internal standard.

Trikatu churna is well known ayurvedic formulation, comprised of the fruits of three medicinal important plants *Piper longum* (Pippali), *Piper nigrum* (Marica) and rhizomes of *Zingiber officianalis* (Saunth), Trikatu churna is a digestive tonic for the assimilation of other foods in the body. It is also used as a rejuvenator and stimulant. Trikatu plays an essential role in the treatment of a wide variety of conditions. It eliminates the aggravated kapha in the respiratory tract and in the digestive channels. It also regulates the path for vata and helps minimize gas formation in the abdomen, being hot in nature[3,4].

The present study is an attempt to develop the fingerprint method for Trikatu churna by 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

Dried fruits of *Piper longum* (Pippali), *Piper nigrum* (Marica) and rhizomes of *Zingiber officianalis* (Saunth) were procured from local market Raipur, India and identified on the basis of morphological and microscopical characters and compared with standard Pharmacopoeial Monograph[5-8].

All the chemicals and solvents were used of AR Grade. Standard Piperine (98 %) was procured from Lancaster England.

Preparation of Trikatu churna: Trikatu churna, three batch name TK-I, TK-II, TK-III, were prepared in laboratory using method described in ayurvedic formulary[3]. These three batches of Trikatu 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 *Zingiber officianalis* (Saunth) does not contain piperine is not included in present study.

Preparation of piperine extract of Trikatu churna: Reflux the powdered Trikatu 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 Trikatu 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. 2 mL of this solution was diluted with ethanol up to 100 mL in volumetric flask to give 20 mg/mL piperine solution.

Calibration curve from standard solution of piperine was prepared and with the help of this curve the piperine of Trikatu 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 342.5 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 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 Fig. 1).

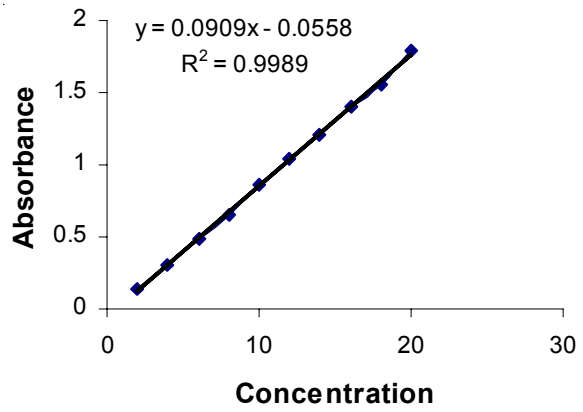

Fig. 1. Calibration curve of piperine

TABLE-1
OPTICAL CHARACTERISTICS, STATISTICAL REGRESSION DATA AND VALIDATION PARAMETER OF PIPERINE

Parameter	Value
Absorption maxima	342.5 nm
Beer's Law limit	2-20 µg/mL
Regression equation (Y = bx + a)	y = 0.0909x - 0.0558
Intercept (a)	0.909
Slope (b)	0.0558
Correlation coefficients (r^2)	r^2 = 0.9989
Precision (n = 6, RSD %)	0.351
Accuracy (%)	99.23

Estimation of piperine: The appropriate aliquots from piperine extract of each batch of Trikatu 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 342.5 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 (Table-2)

TABLE-2
ESTIMATION OF PIPERINE CONTENT IN TRIKATU CHURNA

Name		Piperine content (% w/w)	Confidence level (95 %)
Piper longum (Pippali)		1.40 ± 0.41	± 0.495
Piper nigrum (Marica)		3.60 ± 0.37	± 0.269
	TK-I	1.66 ± 0.61	± 0.528
Trikatu churna	TK-II	1.71 ± 0.49	± 0.426
	TK-III	1.69 ± 0.53	± 0.824

Mean ± SD of six determinations, TK-I: Trikatu Churna Batch I,
TK- II: Trikatu Churna Batch II, TK-III: Trikatu 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 Trikatu churna, of which the piperine content have been estimated previously. The data were obtained and recovery was calculated (Table-3).

TABLE-3
COMPILATION OF DATA OF RECOVERY STUDY

Amount of piperine (µg/mL)			RSD (%)	SE	Recovery (%)
In sample	Added	Estimated			
100	50	149.02 ± 0.61	0.409	0.250	99.34 ± 0.26
100	100	198.23 ± 0.58	0.292	0. 237	99.11 ± 0.24
Mean			0.351	0. 244	99.23

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

RESULTS AND DISCUSSION

Piperine obeys Beer Lambert's law in concentration range 2-20 µg/mL at λ_{max} 342.5 nm. The correlation coefficient (r^2) was calculated where the r^2 value 0.9989 indicates the good linearity between the concentration and absorbance.

The estimation of piperine content of Trikatu churna (three identical laboratory batch) and powdered *Piper longum* (Pippali) and *Piper nigrum* (Marica) was carried out seperately. The concentration of piperine present

in raw material was found to be 3.6 ± 0.37 % (w/w) in marica and 1.4 ± 0.41 % (w/w) in pippali, respectively and in three identical laboratory batches of Trikatu name TK-I, TK-II, TK-III, was 1.66 ± 0.61, 1.71 ± 0.49, 1.69 ± 0.53 % (w/w) (Table-2) respectively with mean value 1.69 ± 0.54 % (w/w).

In order to obtain precision and accuracy, the recovery study was performed at two levels by adding known amount of piperine with preanalyzed sample of piperine in Trikatu churna. The experiment was repeated six times at both level (Table-3) and result shows 99.34 ± 0.26 and 99.11 ± 0.24 % recovery of piperine at both the level with mean value 99.23 ± 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 0.351 at both the level while the standard error was 0.25 and 0.237 with mean 0.244, 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 Trikatu churna.

As Trikatu 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 Trikatu churna.

ACKNOWLEDGEMENTS

The authors are highly grateful to Director, Institute of Pharmacy, Pt. R.S. University, Raipur and AICTE for providing financial assistance under Research Promotion Scheme (AICTE-RPS).

REFERENCES

1. V. Jain, S. Saraf and S. Saraf, *Asian J. Chem.*, **19**, 1406 (2007).
2. World Health Organization, Quality Control Methods for Medicinal Plants Materials, Geneva, pp. 1-15 (1998).
3. Bhaisajyaratnavali, Chowkhamba Sanskrit Series Office,Varanasi, Sloke, edn. 2, p. 19, 35 (1961).
4. The Ayurvedic Formulary of India, Part I, Govt. of India, Ministry of Health and Family Planning, Dept. of Health, Delhi, edn. 1, p. 110 (1978).
5. Indian Herbal Pharmacopoeia, Regional Research Laboratory Jammu, Indian Drug Manufacturing Association Mumbai, Vol. 2, pp. 93-101, (1999).
6. Quality Standards of Indian Medicinal Plants, Indian Council of Medicinal Research, New Delhi, Vol. 1, pp. 168-172 (2003).
7. P. Mukherjee, Pharmacological Screening of Herbal Drug, Quality Control of Herbal Drug: An Approach to Evaluation of Botanicals, Eastern Publishers (Business Horizontal Ltd.), New Delhi, pp. 755-760 (2002).
8. The Ayurvedic Pharmacopoeia of India, Part I; Vol. 2, Govt. of India, Ministry of health and Family Welfare, New Delhi, edn. 1, p. 133 (1999).

(*Received*: 18 September 2006; *Accepted*: 16 June 2007) AJC-5707