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UV-spectrophotometric method development for estimation of piperine in Chitrakadi Vati

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ABSTRACT

A simple, rapid and precise spectrophotometric method has been developed for the estimation of piperine in Ayurvedic formulation Chitrakadi Vati. Three marketed preparations of Chitrakadi Vati containing black pepper (Piper nigrum) and pippali (Piper longum) from different manufacturer (CV-1, CV-2 and CV-3) were taken in this study to estimate the % w/w of piperine. The % w/w content of piperine in the marketed preparations of Chitrakadi Vati CV-1, CV-2 and CV-3 were found to be 0.1032%, 0.0852% and 0.0898 % w/w respectively. Recovery studies were carried out by standard addition method and the average percentage recovery of the three samples CV-1, CV-2 and CV-3 were found to be 98.51%, 99.12% and 98.92% respectively.

Key words: Piperine, Chitrakadi Vati.

INTRODUCTION

Chitrakadi Vati is one of the best classical formulations for indigestion. Chitraka is the name of fire in Sanskrit. Chitrakadi Vati is best for moderate lack of digestive fire. It increases digestion, and also increases appetite by helping the liver to function better. Chitrakadi Vati is a popular remedy employed frequently for its dual digestive effect. Its unique combination acts as an appetizer if taken before meal and as a digestive if taken after meal. By stimulating fat burn it also helps in mobilizing over deposited fat from the tissues[1]. Chitrakadi Vati increases the Pitta, and regulates Vata in the stomach. It stimulates appetite by provoking digestive fire, restores normal appetite, promotes proper secretion of digestive Pitta. Chitrakadi Vati removes Aama Dosha, which is developed due to lack of fire. Aama leads to formation of endotoxins which is root cause of many auto-immune diseases like rheumatoid arthritis, scleroderma, nephrotic syndrome and ankylosing spondylitis etc. Chitrakadi Vati is useful in irritable bowel syndrome,

anorexia, frequent loose stools, heaviness in abdomen, gas formation. It maintains the peristaltic movements of the intestine, and stops the flow of undigested food along with loose stool[2].

Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological activities, higher safety margins and lesser costs. In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is about $ 80 million[3]. The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. But the most important challenges faced by these formulations arises because of their lack of complete standardization. Commercialization of the manufacture of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine[4]. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration, variation in composition and level of active constituents due to variation in climatic conditions. Also variation in the chemical profile of the herbal formulations is due to the factors like growing, harvesting, storage and drying processes. Therefore quality control of herbal medicines offers a host of problems[5-8]. It is very important that a system of standardization is established for every plant medicine available in the market because the scope for variation in different batches of medicine is enormous. The main problem with polyherbal formulation is that it consists of a large number of phytoconstituents and each constituent has to be determined that is not an easy task. Also presence of large number of ingredients in a formulation may cause interference in determination of each other. The present study is based on the development of a simple spectrophotometric method for the estimation of piperine in Chitrakadi Vati. Piperine is 1-[5-(1, 3-Benzodioxol-5-yl)-1-oxo- 2, 4-pentadienyl] (**Figure 1.**), an active constituent of the black pepper (*Piper nigrum*) and pippali (*Piper longum*) having potent anti-tussive and bronchodilator properties.

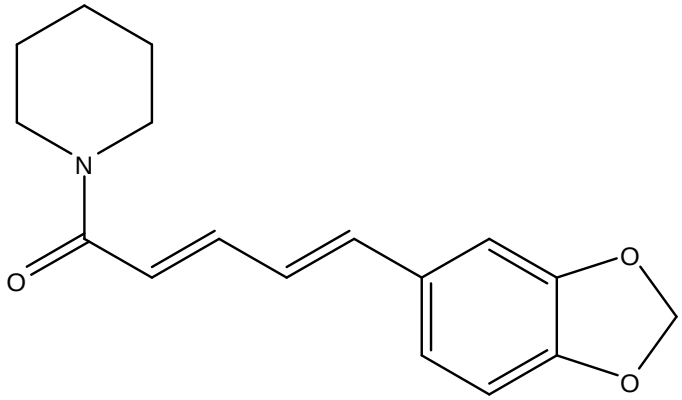

Figure 1. Chemical structure of Piperine

MATERIALS AND METHODS

Piperine standard was isolated from the fruits of *Piper nigrum*. Spectroscopic grade methanol and ethanol were purchased from Merck India Ltd. UV absorbance was recorded using Varian UV-spectrophotometer with Carry-100 software.

Preparation of standard solution for calibration curve of piperine

Stock solution of piperine was prepared by dissolving 10 mg of piperine in 100 ml of methanol. Standard solutions of piperine were prepared from stock solution in the concentration range of 2-20µg/ml in 100 ml volumetric flask using methanol as solvent. The absorbance of piperine standard solutions was measured at 342 nm (λ_{max} for piperine, **Figure 2**.) against methanol as blank. Calibration curve was plotted between absorbance and concentration (**Figure 3**.).

Table 1. Drug ingredients used in the preparation of Chitrakadi Vati

Crude Drugs	Botanical Name
Chitraka	*Plumbago zeylanica*
Pippali	*Piper longum*
Yavakshar	-
Sajjikshar	-
Rock salt	-
Saunchar salt	-
Black salt	-
Samudra salt	-
Sambhar salt	-
Shunthi	*Zingiber officinale*
Maricha	*Piper nigrum*
Hingu	*Ferula narthex*
Ajmoda	*Carum roxburghianum*
Chavya	*Piper retrofractum*

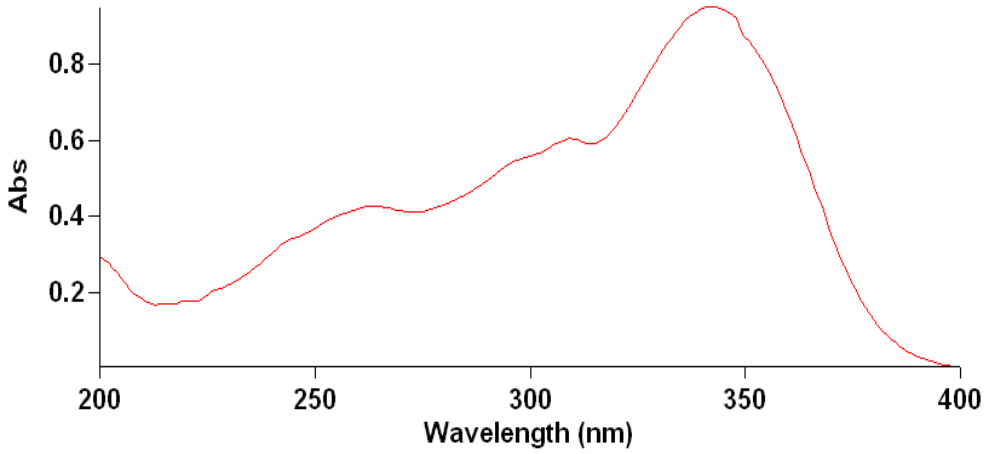

Figure 2. UV absorption spectra of Piperine

Preparation of sample solution

Three marketed formulations of Chitrakadi Vati from different manufacturer (CV-1, CV-2 and CV-3) were taken in this study to estimate the % w/w of piperine. Of each sample 5 gm were taken and converted into powdered form. Each sample were transferred separately in 100 ml round bottom flask and refluxed with 100 ml of ethanol for 1 hour. The extract was filtered and re-refluxes the marc left with 50 ml of ethanol for another 1 hours. Filtrates were combined and subjected to concentration in rotary evaporator. Residues obtained were dissolved in methanol

and volume made up to 1000 ml with methanol. The absorbances of sample solutions were measured at 342 nm against methanol as blank. Same procedure was repeated for two different days.

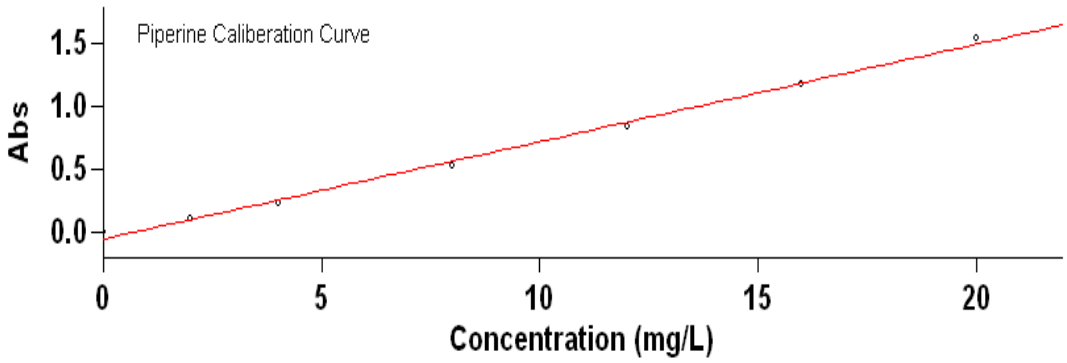

Figure 3. Calibration curve of piperine

RESULTS AND DISCUSSION

The method involves measurement of UV absorbance at 342nm for piperine corresponding to the absorption maxima of the herbal formulations. The absorbance characteristics showed that piperine obeys Beer Lambert's law within the concentration range 2-20 µg/ml at the λ-max of 342 nm with the regression value of 0.9956 and calibration equation Y = 0.07733*X -0.04974 (**Table 2**). The % w/w content of piperine in the marketed preparations of Chitrakadi Vati CV-1, CV-2 and CV-3 were found to be 0.1032%, 0.0852% and 0.0898 % w/w respectively (**Table 3**). Almost similar results were obtained for two days of all three samples with %RSD value less than 0.12, indicating the reproducibility of the method.

Table 2. Table for standard curve of Piperine

Standard	Conc. (µg/ml)	Mean[a]	SD	%RSD
Std.1	0.0	0.0001	0.0000	0.00
Std.2	2.0	0.1148	0.0003	0.22
Std.3	4.0	0.2378	0.0006	0.25
Std.4	8.0	0.5261	0.0007	0.16
Std.5	12.0	0.8248	0.0003	0.06
Std.6	16.0	1.1797	0.0004	0.07
Std.7	20.0	1.5453	0.0006	0.09

[a]*Values expressed as mean of three readings*

Recovery studies were carried out by standard addition method and the average percentage recovery of the three samples CV-1, CV-2 and CV-3 were found to be 98.51%, 99.12% and 98.92% respectively. Results obtained from the recovery study indicating the accuracy and precision of the method.

Table 3. Table for Piperine content in the samples of Chitrakadi Vati

Sample		Conc. (µg/ml)	Mean[a]	SD	%RSD
CV-1	1st day	5.16	0.3495	0.0002	0.05
	2nd day	5.16	0.3493	0.0001	0.03
CV-2	1st day	4.26	0.2798	0.0004	0.12
	2nd day	4.26	0.2800	0.0002	0.08
CV-3	1st day	4.49	0.2982	0.0003	0.06
	2nd day	4.49	0.2979	0.0001	0.08

[a]*Values expressed as mean of three readings*

CONCLUSION

The developed method was found to be accurate, simple, precise and rapid. It can therefore be applied for routine analysis of piperine in polyherbal formulations containing piperine.

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