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A SPECTROPHOTOMETRIC METHOD TO ESTIMATE PIPERINE IN PIPER SPECIES

S.K. CHAUHAN, G.P. KIMOTHI, B.P. SINGH AND S. AGRAWAL
R& D Laboratory, Indian Herbs, P.B.No.5, Sharda Nagar, Saharanpur – 247

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ABSTRACT: *A Simple, rapid and economical procedure for estimation of piperine by UV Spectrophotometer in different piper species was developed and is described. The method is based on method is based on the identification of piperine by TLC and on the ultra violet absorbance maxima in alcohol at 328 nm.*

INTRODUCTION

Piper nigrum and *Piper longum* are most widely used, medicinally important species of the family piperaceae which are aromatic and possess stimulant, pungent, carminative and antiseptic properties, Numerous uses of these species have been mentioned in the Ayurvedic and Unani system of medicine[1,2 & 3]. Piperine is considered to be the most active ingredient present in *P. nigrum, P. longum* etc. in varying amount. The importance of piperine has been highlighted in recent ears because of its role in the enhancement of bioavailability [4&5] of certain other drugs. However, the precise and sensitive analytical methods to determine piperine are scanty and grvimetric[6] and titrimtric[3] methods ma e found in the literature. These methods may be found in the literature. These methods are not very precise and are cumbersome to perform. For example, they involve multiple step extraction and hydrolysis. We have developed a precise, sensitive and reproducible methods for estimation of piperine in *P. nigrum* and *P.longum*. This method can be used to standardize these plant materials with reference to piperine

MATERIALS AND METHODS

Instrument

UV visible recording spectro-photometer (Beckman DU 64)

Standard preparation

A 0.20 mg/ml solution of pipe5ine reference standards (Sigma, USA) was prepared in methanol. It was further diluted to yield the final concentration of 0.004, 0.005, 0.008 and 0.016 mg/ml.

Sample preparation

Around 25gm air dried samples of *P.nigrum* and *P. longum* were taken and ground separately to pass through 40 mesh S.S. Sieve. 1 gm sample from each was accurately weighed and transferred to 100ml conical flasks. 15 ml alcohol was added to the respective flasks for extraction the extraction was performed over steam water bath by shaking frequently and heating the flasks till the solvent starts boiling. The were allowed to remain in boiling condition for 4-5 minutes, which were then filtered using whatman filter paper no. 41 in hot condition. The above extractions were repeated four more times using 15ml of

alcohol each time the clear filtered extracts were quantitatively transferred to 100ml volumetric flasks. The filter papers were washed with to alcohol (5mlx2) and transferred to respective volumetric flasks.

The volume of extracts was adjusted upto the mark with alcohol. The volumetric flasks were then shaken by stoppering to make the contents homogenous. 1.0ml of *P. longum* extract was exactly pipetted our into a 20 ml capacity volumetric flask; the volume was made up to mark with alcohol, shaken well and used further.

In the case of *P. nigrum* extract, 5.0ml of extract was exactly pipetted out into a separate 100ml volumetric flask. The volume was made up to the mark with alcohol and shaken well to make the contents homogenous. These solutions were further used for spectrophotometry.

Identification by TLC

For TLC identification the parent sample i.e. 1 gm in 100 ml was used as such without an further dilution. 10ul of each test sample along with 10 ul of standard piperine were applied on precoated silica gel 60F 254 aluminium plate. The chromatogram was developed in the solvent system, Toluene: Ethyl acetate = 70:30 upto 80 mm.

The plate was observed under UV 254 nm (Fig.1) which is then sprayed wit Dragendroff's reagent.

Procedure

The absorbance of four different concentrations of standard piperine was measured at 328nm. The calibration curve was plotted between concentration and absorbance. The linear equation from the calibration curve was plotted which was found to be Y = 0.267 +100.28 x with a correlation coefficient of 0.999 where Y is the absorbance and x is the concentration in mg/ml. The absorbance of test samples was also measured at 328nm and the concentration was adjusted to give the absorbance between 0.5 to 1.0. The amount of piperine in test samples was determined using the above equation (Table -1).

Recovery Studies

A varying known amount of standard piperine was added to the crude drugs viz *P. nigrum* and *P. longum*. The samples were processed and analysed as per the procedure mentioned above. The results are mentioned in table -2.

RESULTS AND DISCUSSION

Under the chromatographic condition mentioned above. The Rf of piperine was observed at about 0.2 which also provides Dragendroff's positive reaction. The calibration curve was found to be linear between 4.0 to 16 µg. The method allows reliable quantification of piperine from different piper species. Further, recover values were also satisfactory which sowed the reliability and suitability of the sowed. The proposed methods is rapid, simple and accurate and hence can be used for quantitative monitoring of piperine in different *piper* species.

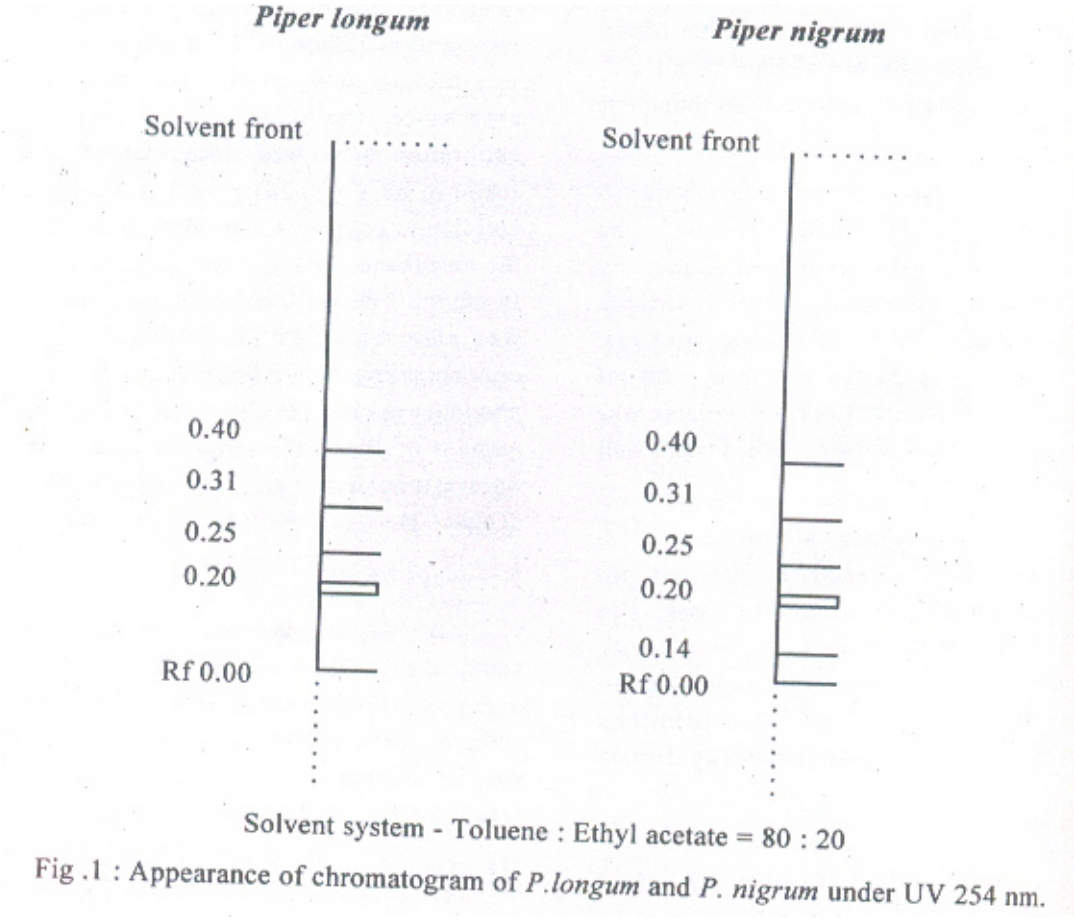

Fig .1 : Appearance of chromatogram of *P.longum* and *P. nigrum* under UV 254 nm.

Table No1
Estimation of Piperine

S. No	Details of sample	% of Piperine on dry weight basis
1.	*Piper nigrum*	5.014
2.	*Piper longum*	1.434

Note – Each value is the average of three replicates

Table 2
Method validation and recovery of Piperine

S.No	Details of samples	Amount of sample taken (mg) (A)	Amount of piperine present in A (mg (B)	Amount of piperine added to A (mg) (C)	Amount of total piperine taken (mg) D= (B=C)	Amount of piperine found (mg) (E)	% recovery E/D x 100
1.	*Piper nigrum*	1010	50.64	5.00	55.64	55.05	98.94
2.	*Piper nigrum*	1050	52.65	10.00	62.65	61.95	98.88

3.	*Piper nigrum*	1040	52.14	15.00	67.14	67.00	99.79
4.	*Piper longum*	1010	14.48	5.00	19.48	19.18	98.45
5.	*Piper longum*	1040	14.91	10.00	24.91	24.63	98.87
6.	*Piper longum*	1050	15.06	15.00	30.06	30.10	100.13

Average percentage recovery – 99.18 %

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