ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Status of piperine content in Ayurvedic formulation: Method standardization by HPTLC

Vishvnath Gupta and UK Jain *

Bhopal Institute of Technology & Science-Pharmacy, Bhopal (M.P)

ABSTRACT

Ajmodadi churna (AJC) is an Ayurvedic formulation containing Piper species (Piper longum in both form root and fruit and Piper nigrum) as main ingredients. It is a traditional Ayurvedic oral Herbal formulation, available as a popular proprietary, from most manufacturers of ayurvedic drugs. A selective, precise and accurate High Performance Thin Layer Chromatography (HPTLC) method has been developed for the simultaneous quantification of Piperine in Ajmodadi churna as well as its bulk drug. The method employed TLC aluminum plate precoated with silica gel 60 F_{254} as a stationary phase. The solvent system consists of Toluene: Ethyl acetate(7 : 3 % v/v). This system was found to give compact spot for Piperine. Densiometric analysis was carried out in the absorbance mode at 254 nm. The linear regression analysis data for the calibration plot showed good linear relation with r^2 = 0.992 and with respect to peak area for Piperine , in concentration range 0.5-20 µg/spot. The method was validated for precision (0.173), recovery (99.43%), Limit of Detection (0.063mg/ml) and Limit of Quantification (0.071mg/ml). The proposed HPTLC method can be used for the quality control of the raw materials as well as formulations. Piperine in *Piper longum* and *Piper nigrum* are reported to be the active components in the formulation AJC and can be considered as marker compounds. Therefore, HPTLC method has been developed for the estimation of these marker compounds [11], and also to develop finger print profile for the standard formulation of Ajmodadi churna so that these parameters can be compared with any marketed formulation for evaluating its purity and quality. The proposed method has been validated as per ICH guidelines.[12-13]

Keywords: Piperine, HPTLC, ayurvedic formulation (Ajmodadi churna), validation.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Standardization and analysis of the chemical marker of the Ayurvedic and other poly herbal formulation is always very big problem. Quantitative estimation of chemical markers of each ingredient in the poly herbal preparation required ideal separation technique by which these markers are separated with highest purity and with least inferences from each other [1]. For botanicals and herbal preparations, there is a requirement for scientific proof and clinical validation with chemical standardization, biological assays, animal models and clinical trials [2].

Ajmodadi churna used in abdominal pain, carminative, anti-spasmodic, and helps in all painful conditions like sciatica and stiffness. Ayurvedic formulary of India has given the specification for the composition of AJC, it should contain piper species as a major ingredient apart from different herbs and salts [3,4].

In the present study, we report the development of a simple, optimized and validated HPTLC method for the simultaneous estimation of Piperine in AJC. Piperine chemical marker was selected, one from each medicinal herbs used as raw materials, Piperine from Pippali, for the quantification purpose because these markers are responsible for the physiological action of the plants [5,6]. The method was validated on the basis of its selectivity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) according to ICH requirements [9].

MATERIALS AND METHODS

Equipment

The instrument used for estimation , was Camag Linomat V semiautomatic sample applicator, CAMAG TLC Scanner "Scanner_170422" S/N 170422 (2.01.02), CATS V.4.06 software for interpretation of the data Hamilton syringe and Camag twin through chamber.

Chemicals

Standard Piperine was purchased from Total herbs solutions Pvt. Ltd. (Mumbai, India). Analytical grade reagents; n-hexane, ethyl acetate, acetone, formic acid and methanol (Merck Chemicals, India) were used. Stationary phase used was Silica gels G60F254, 10 x10 cm TLC plate 0.2mm thickness were obtained from E. Merck Ltd (Mumbai, India).

Drugs

Crude drugs were procured from local market and identified by macroscopic and microscopic characters[7,8] and authenticated by the Department of Botany, Harisinghgour University, Sagar(M.P.) .

ISSN: 0975-8585

Preparation of formulations

1. Three batches were prepared in laboratory (named as AJC-I,AJC-II and AJC-III) according to strict methods of 'Ayurvedic formulary of India' and Sarangadhara-samhita.

2. Commercially available brands AJC-A, AJC-B, and AJC-C, of Ajmodadi churna were procured from local market.

HPTLC method for estimation of Piperine

Preparation of standard piperine solution

A stock solution of piperine, 1 mg/ml was prepared by dissolving 10mg of accurately weighed piperine in methanol and making up the volume to 10 ml in amber coloured volumetric flask covered with aluminium foil, because piperine in solution isomerizes to isopiperine, chavicine and isochavicine on exposure to light[10]. The stock solution was further diluted with methanol to yield a concentration of 100µg/ml of piperine.

Preparation of Sample solution

The laboratory formulation samples and the commercial formulation samples (1gm each) of Ajmodadi churna were extracted using maceration process in 10ml methanol. This extract is filtered through a whatmann filter paper no.1 and the filtrate collected in a 100 cm^3 volumetric flask, and then solicited the solution. The contents are diluted to volume with methanol, this solution gives the final concentration of 20 mg/ml.

Chromatographic conditions

Stationary phase : HPTLC precoated, silica gel G60, F_{254}(Merck)
Thickness : 0.2 mm
Mode of application : Band
Band width : 8mm
Separation technique : Ascending
Temperature : 25 ± 3°
Saturation time : 15 min with plate
Migration distance : 80 mm
Wavelength : 336 nm (CAMAG 3Scanner)

Preparation of Mobile Phase

Mobile Phase was prepared by mixing Toluene and ethyl acetate in the proportion of 07 :03 v/v.

ISSN: 0975-8585

Calibration Curve

Standard solution of Piperine concentration of 100 µg/ml prepared and was applied duplicate in 1_L, 2_L, 3_L, 4_L,5_L, 6_L, 7_L, 8_L, 9_L, 10_L over the silica gel G 60F $_{254}$, plate. The plate was developed and analyzed as described ,earlier. The plate was then developed using the optimized mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Validation of method

The developed method was validated in terms of Linearity, Accuracy, and precision, Limit of detection, Limit of quantification, robustness and ruggedness [5,6].

RESULTS AND DISCUSSION

The solvent system of Toluene: ethyl acetate (07:03 v/v) was found to be ideal mobile phase for separation of piperine. Standard piperine showed single peak in HPTLC chromatogram (Figure II). Calibration curve of piperine was prepared by plotting concentration of piperine versus average area of the peak. The methanol extract of formulations shows more number of peaks. The difference of % it may be due to varied factors like drug variety, geographical variation, and age of the plant at the time of harvest, genetic and environmental factors.

Quantitative Estimation

In the chromatogram of the drugs extracted from the churna, many well resolved spots were observed, out of these spots one spot was matches with the Rf value (0.42± 0.01) shown by Standard Piperine and having the same lambda max (343nm) (Figure II – Table-III). The drug content as per label claim of laboratory formulation (AJC-I, AJC-II and AJC-III)was found to contain 0.50±0.006 %, 0.51±0.002% and 0.53±0.005%w/w of piperine. A while the marketed formulations (AJC-A,AJC-B and AJC-C) shows 0.40±0.001 %, 0.41±0.008% and 0.45±0.002%w/w of piperine with % Residual Standard Deviation (R.S.D) is 0.173%(mean). The low %R.S.D values as shown in (Table-III) indicated the suitability of this method for routine analysis of Piperine in Ayurvedic formulation (AJC).

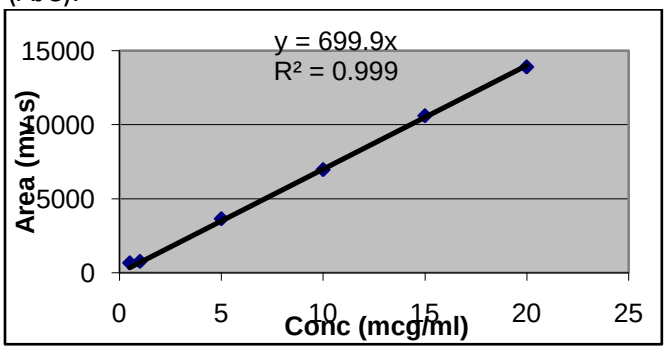

Figure I: Calibration Curve of Piperine

ISSN: 0975-8585

Calibration Curves

Calibration graph was found to be linear over the concentration range 0.5-20 ug/spots. The peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation Y=699.9X and regression coefficient [3,4] (r^2) was 0.999 and R.S.D. was found 0.173(Figure -I). Response obtained for piperine in preparation of calibration curve (Table –III).

Linearity

A representative calibration curve of piperine was obtained by plotting the peak area of piperine against the concentration of piperine (0.5-20ug/ml) respectively. The correlation coefficient for piperine was found to be 0.999 respectively and thus exhibits good linearity between concentration and area.

Accuracy (Recovery Studies)

To study accuracy of the developed method, recovery studies were carried out using standard addition method at three different level and the % recoveries were calculated. The average % recovery of laboratory formulation was found to be 99.10 % and marketed formulation was found to be 99.77 % which are satisfactory.(Table II).

Specificity

It was observed that other constituents present in the formulations did not interfere either with the peak of piperine (Figure-II). Therefore the method was specific. The spectrum of standard piperine and piperine present in the samples were found to be similar or overlap. (Figure-III and Figure -IV)

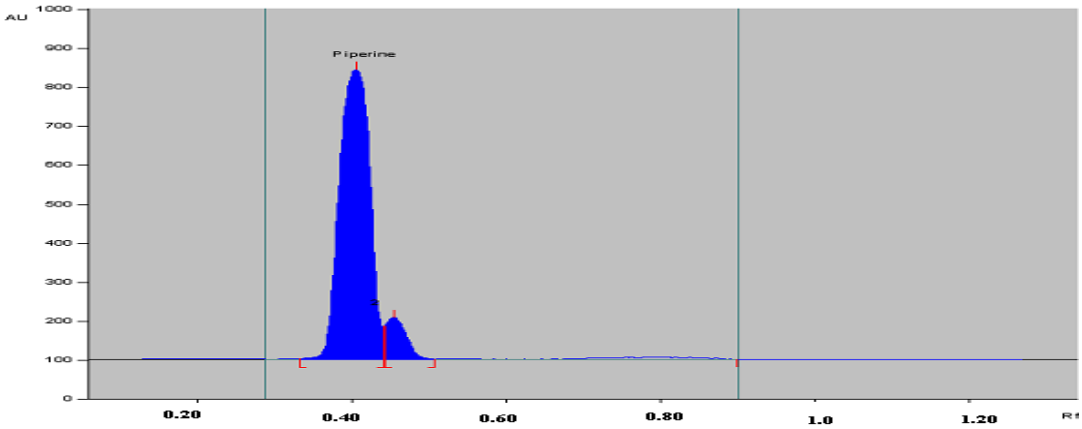

Figure II: Peak response of STD Piperine

ISSN: 0975-8585

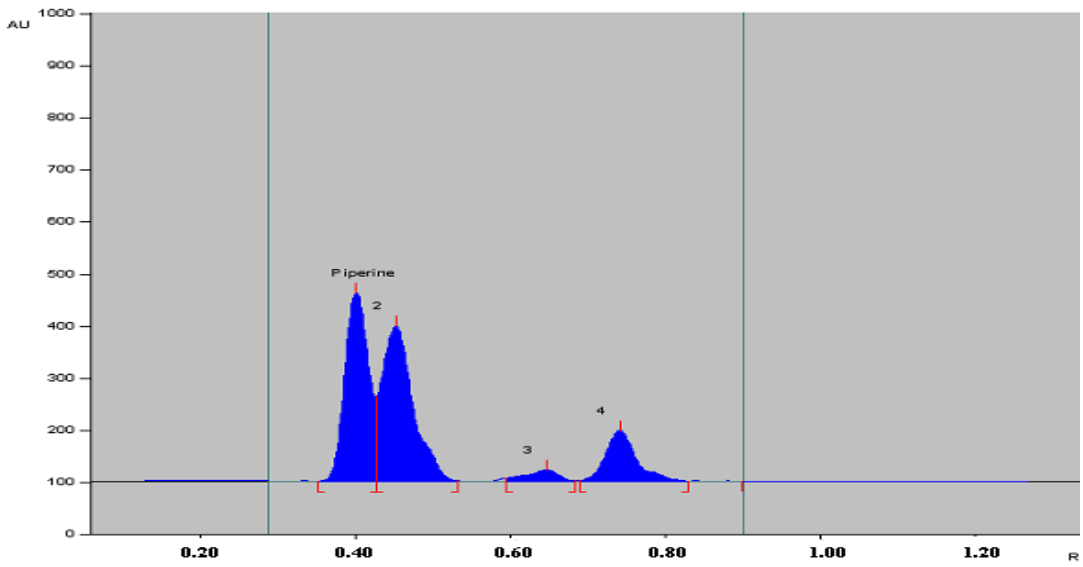

Figure III: Peak response of Ajmodadi churna marketed

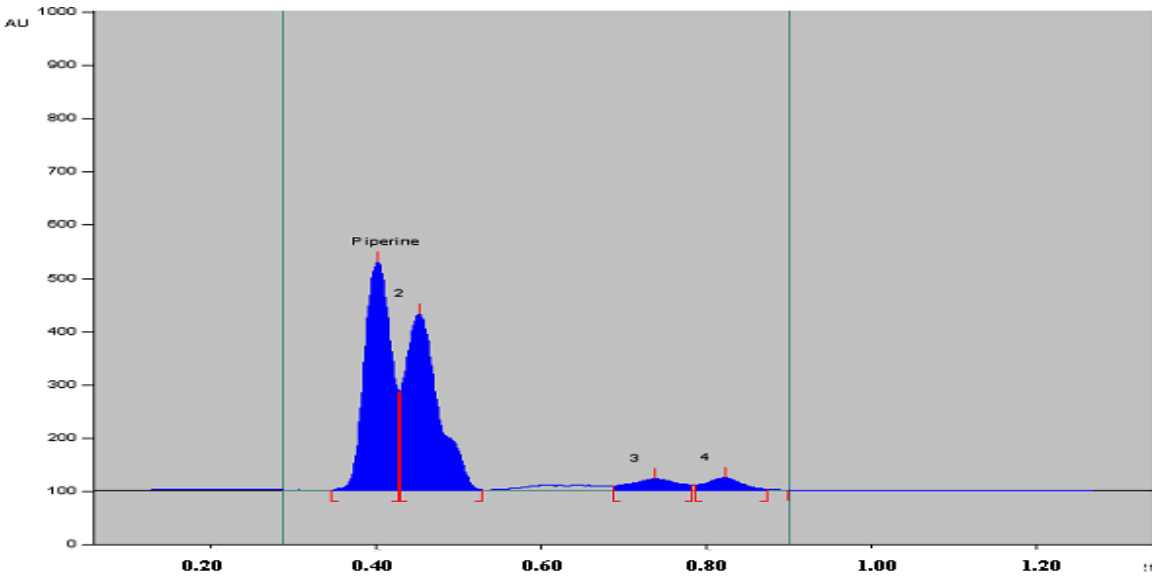

Figure IV: Peak response of Ajmodadi churna prepared in Lab

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % R.S.D. Precision studies were carried out by using the sample solution. The amount of Piperine present in per track were calculated by using regression equations . The results were revealed that the % R.S.D. was found to be < 2% i.e. (0.173% mean) (Table II).

ISSN: 0975-8585

Table I: Estimation of piperine (Mean% ± SD, n=3):

S.NO.	NAME		PIPERINE CONTENT%W/W
01	piper longum(pippli) piper longum(pippalimula) piper nigrum(marica)		1.45±0.34 1.63±0.21 3.97±0.16
02	AJMODADI CHURNA	AJC-I	0.50±0.006
03		AJC-II	0.51±0.002
04		AJC-III	0.53±0.005
05		AJC-A	0.40±0.001
06		AJC-B	0.41±0.008
07		AJC-C	0.45±0.002

Table-II: Data of recovery study (Mean% ± SD, n=3):

S.NO.	AMOUNT OF PIPERINE(MICROGM/ML)			RSD%	SE	RECOVERY%
	IN SAMPLE	ADDED	ESTIMATED			
01	100	100	198.2±0.62	0.312	0.21	99.10
02	200	150	349.2±0.12	0.034	0.024	99.77
mean				0.173	0.117	99.435

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

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the amount of applied sample producing the peak area which is equal to the sum of the mean blank area and three times of its standard deviation.LOQ is the amount of applied sample producing the peak area which is equal to the sum of the mean blank area and ten times of its standard deviation. The LOD and LOQ were found to be 0.063mg/ml and 0.071mg/ml respectively. (Table III).

Table-III Validation parameters of piperine (Mean% ± SD, n=3):

S.No.	parameter	value
1	Absorption maxima	343
2	Bee's law limit	0.5-20ug/ml
3	Regression equation(y=bx+a)	699.9x
4	Intercept(a)	0
5	Slope(b)	699.9
6	Correlation coefficients(r^2)	0.999
7	LOD mg/ml	0.063
8	LOQ mg/ml	0.071
9	Precision (n=6, % RSD)	0.173
10	Accuracy (%)	99.43

Ruggedness and Robustness

ISSN: 0975-8585

The study of ruggedness and Robustness was carried out by keeping all the parameters constant except for the time, day and analysts. The results were shown in (Table IV). All the Validation study data were shown in (Table III).

Table-IV Different parameters of ruggedness & robustness

Serial no.	Parameters	Initial condition	Changed condition	Effects
1.	Mobile Phase composition	Toluene : Ethyl acetate (7:3)v/v	Toluene : Ethyl acetate (7:3)v/v	Rf increased but no effect on resolution.
2.	Development Distance	80mm	60mm	No effect on resolution.
3.	Temperature	25	40	Number of bands increased
4.	Tank Saturation Time	15min	30min	Plate develops slowly.
5.	Extracting solvent	Methanol	Chloroform	No effect on resolution.

CONCLUSION

The proposed HPTLC method was found to be rapid ,simple and accurate for quantitative estimation of Piperine in different formulation extracts. The % recovery values of piperine was found to be 99.435% , which shows the reliability and suitability of the method. The standard deviation and coefficient of variation were 0.173 and 0.999 respectively. Thus proving the accuracy and precision of the analysis. The method was found to be useful in detecting the genuine of the formulation and thus suitable to evaluate various formulations available in the market. The proposed solvent system and the scanning wavelength found suitable to identify and estimate piperine respectively.

ACKNOWLEDGEMENT

The authors are grateful to Principal, BITS-Pharmacy, Bhopal for their unforgettable support.

REFERNCES

[1] Mukherjee PK. Quality Control of Herbal Drugs. New Delhi: Business Horizons, India; 2005. P.741.
[2] Ong ES. J Chromatogr B. 812:23-33.
[3] The Ayurvedic Formulary of India. Part-I, New Delhi: Government of India, Ministry of Health and Family Welfare; 2000, 2nd Ed, 31.
[4] The Ayurvedic Pharmacopoeia of India. Vol. I, New Delhi: Government of India, Ministry of Health and Family Welfare; 2001, 1st Ed. 162.
[5] Indian Herbal Pharmacopoeia. Mumbai: Indian Drug Manufacturer's Association, 2002; p.306- 315.

[6] Malhotra SC. Phytochemical investigations of Certain Medicinal Plants Used in Ayurveda Yugantar Prakasham. 1990. 175.
[7] Quality Standard of Indian Medicinal Plants., New Delhi: Indian Council of Medicinal Research; 2003, Vol 3, 1.
[8] Quality Standard of Indian Medicinal Plants., New Delhi: Indian Council of Medicinal Research; 2003, Vol. 1, 168-172.
[9] Guideline on Validation of Analytical Procedure- Methodology. Geneva; International Conference on Harmonization; 1996.
[10] Agrawal SS and Pardhavi M. Herbal Drug Technology. Universities press. Hyderabad. 2007; 361-363.
[11] Sethi PD. High Performance Thin Layer Chromatography. 1st Edition. New Delhi, India: CBS Publishers and Distributors; 1996. pp. 3–71.
[12] ICH, Q2A, author. Proc Int Con Harmonization. Geneva: 1994. Validation of analytical procedure: Methodolo.
[13] ICH, Q2B, author. Proc Int Con Harmonization. Geneva: 1996. Validation of analytical procedure: Methodolo.