Pharmacogn Res. 2022; 14(2):219-224
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogres.com | www.phcog.net

Original Article

A Novel Stability Indicating Method for Determination of Major Alkaloid in Black Pepper by RP-HPLC in Different Pharmaceutical Dosage Forms

Yenduri Suvarna[1,*], Shaik Abdul Rahaman[2], Arram Madhavi[3]

ABSTRACT

Background: Piperine is the major alkaloid found in the fruits of Black pepper. Recent studies revealed the antiurolithiatic effect of piperine. So, an attempt was made to develop an analytical method for the assay of Piperine in the formulated dosage forms. **Objectives:** The present study was done with the aim of developing a simple, accurate, precise and sensitive RP-HPLC method for estimation of Piperine in different dosage forms. **Materials and Methods:** Some trials were performed during method development using different solvents, mobile phase compositions and flow rate for the estimation of piperine in the dosage form. The developed optimized method was validated as per ICH guidelines and was employed to estimate the amount of piperine in the given dosage form. **Results:** The optimized chromatographic conditions were achieved using BDS C8 column with mobile phase having of water: Acetonitrile in 50: 50 ratio at 1.0ml/min flow rate. Detection was observed at 247nm using PDA detector. The retention time obtained for piperine peak was found to be 2.4 min. **Conclusion:** The analytical method which was developed for estimation of piperine is simple, rapid, economic, specific, precise, stable and can be successfully employed for its estimation in syrup and tablet dosage forms.

Keywords: Piperine, Tablet and syrup dosage forms, RP-HPLC, Validation, ICH guidelines.

Yenduri Suvarna[1,*], Shaik Abdul Rahaman[2], Arram Madhavi[3]

[1]Department of Pharmaceutical Chemistry and Analysis, Sri Adichunchanagiri College of Pharmacy, B.G. Nagar, Karnataka, INDIA.
[2]Department of Pharmaceutical Analysis, Nirmala College of Pharmacy, Atmakur, Mangalagiri, Guntur, Andhra Pradesh, INDIA.
[3]Department of Pharmaceutical Analysis, Geethanjali College of Pharmacy, Keesara, Telangana, INDIA.

Correspondence

Dr. Yenduri Suvarna

Department of Pharmaceutical Chemistry and Analysis, Sri Adichunchanagiri College of Pharmacy, B.G. Nagar-571448, Karnataka, INDIA.
Email id: suvarna.ph52@gmail.com
ORCID iD: 0000-0002-2928-1446

History

- Submission Date: 19-03-2022;
- Review completed: 04-04-2022;
- Accepted Date: 20-04-2022.

DOI : 10.5530/pres.14.2.31

Article Available online
https://www.phcogres.com/v14/i2

INTRODUCTION

Piperine is an yellow to pale white crystalline powder with pungent taste. It is an alkaloid substance obtained from dried unripe fruits of the plant *Piper nigrum* belonging to the family *Piperaceae*.[1] It is hydrophobic molecule with a log p value of 2.7,[2] and its Pk_a value is 13.2,[3] which indicates that it is very weak acid. Chemically it is 1-piperoylpiperidine (or) (2*E*, 4*E*)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one,[4] and is shown in Figure 1. Piperine shows multiple pharmacological activities like anticancer, anti-inflammatory, antioxidant, antihypertensive. Recently, it was found that piperine also shows antiurolithiatic activity,[5-6] Several methods are available for estimation of Piperine in its extract,[7-9] polyherbal formulations,[10-13] but no method was found for its estimation in tablet and syrup dosage form. The main objective of this work is to estimate the amount of piperine in tablet and syrup dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Piperine, purchased from Shreedha phytoextracts pvt ltd, Jaipur. HPLC grade water, HPLC grade acetonitrile, Orthophosphoric acid (OPA) were supplied from Thermofisher Scientific.

Instrumentation

Development and validation of a method for the assay were performed on HPLC (Waters 2690), PDA detector with empowering 2 software. Hypersil BDS C_8 (150mm x 4.6mm x 5.0 m) reverse phase column was used for chromatographic separation.

Methods

Different trials were performed during method development using different columns, buffers, mobile phase compositions for the estimation of piperine in tablet and syrup dosage form. As piperine is a photosensitive drug, ambered coloured glassware was used.

Preparation of Tablet formulation[14]

As Piperine is slightly soluble in water, a solid dispersion was formulated by solvent evaporation method to enhance its solubility using HP β Cyclodextrin as a polymer in 1:1 ratio with methanol as solvent. Piperine tablet was prepared

Cite this article: Suvarna Y, Rahaman SKA, Madhavi A. Novel Stability Indicating Method for Determination of Major Alkaloid in Black Pepper by RP-HPLC in Different Pharmaceutical Dosage Forms. Pharmacog Res. 2022;14(2):219-24.

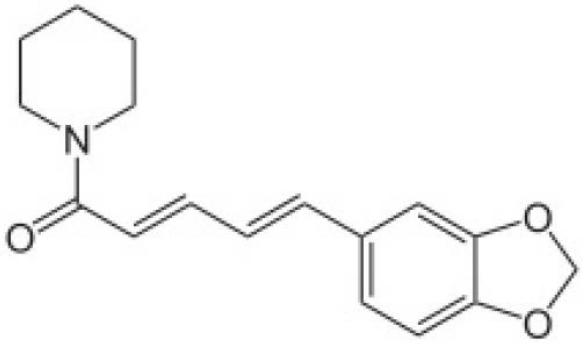


Figure 1: Chemical structure of Piperine.

by direct compression by taking the equivalent weight of Piperine and suitable excipients. All the ingredients were passed through # 60 mesh sieve separately. Piperine and Microcrystalline cellulose were mixed thoroughly by adding small quantities each and properly blended to get a homogenous mixture. Then other excipients like sodium starch glycolate (SSG), talc, magnesium stearate were mixed in geometrical series and allowed to pass through a coarse sieve (#44 mesh) and the tablets were obtained by compressing in the hydraulic press.

Preparation of Syrup formulation[15]

The sugar base for the syrup was prepared by mixing 85g of sucrose in 45g of water and the mixture heated to its boiling point. After straining the liquid, the volume made up to 100ml with distilled water. The preservatives methyl paraben and propyl paraben were dissolved in a small volume of boiled and cooled water and then it was added to the sugar base. The required amount of Piperine was dissolved in propylene glycol which was heated to 45-50°C and then sorbitol, glycerin was added to it. Finally, sodium saccharin was added and thoroughly mixed. pH was adjusted between 5.5-6.5 with citric acid, if necessary. Then volume was made up to 25ml with boiled and cooled water.

Mobile phase preparation

0.1% OPA buffer was prepared by adding 1ml OPA in a 1000ml of volumetric flask followed by adding 100 ml milli-Q water and mixed thoroughly. Finally, the volume was made up to mark with milli-Q water and it was degassed. Buffer and acetonitrile (50:50%v/v) were employed as mobile phase.

Preparation of Diluent

Equal volumes of water and ACN were mixed and used as diluent for preparing solutions.

Preparation of Standard stock solutions

Piperine of 39mg was accurately weighed and transferred into a volumetric flask of 100ml volume followed by the addition of diluents to half of its volume. The mixture was sonicated for 10 min. Then it was made up to volume with diluent and marked as Standard stock solution (390µg/ml of Piperine)

Preparation of working Standards

Different working standards are prepared by diluting Piperine standard stock solution with diluents.

Preparation of working Standard (100% solution)

From standard stock solution, 1ml was transferred to 10ml volumetric flask and made up to mark with diluent. (39µg/ml of Piperine).

Preparation of Sample stock solutions

For tablets assay, twenty tablets of piperine were weighed accurately and pulverized. An amount of tablet powder equivalent to 39mg of piperine was weighed and transferred into 100ml volumetric flask. 50 ml of the prepared diluent was transferred and sonicated for 25 min. The solution was made up to volume with diluent and filtered through 0.45µ filter (390 µg/ml of Piperine).

For syrup formulation, the sample stock solution was prepared by taking an amount of syrup equivalent to 39mg into a 100 ml volumetric flask, followed by adding 50ml of diluents. The mixture was sonicated for 25 min and the volume was made up with diluents. The solution was filtered by passing through 0.45µ filter (390 µg/ml of Piperine).

Solution stability

The stability of the solution was studied by injecting the standard solution into the instrument immediately after preparation (0 hr) and again injected after 24 hr storage at room temperature. The peaks obtained were compared to understand their stability.

Validation

System Suitability Parameters

The system suitability parameters of the optimized method were assessed by taking standard solutions of Piperine (39ppm) and were injected six times. The system suitability parameters like USP plate count, resolution and peak tailing were determined.

The % RSD of peak areas of six standard injections results should be NMT 2%.

Specificity

It was done for checking interference in the optimized method if any. There should not be any interfering peaks in blank and placebo at the retention times of these drugs in this method.

Linearity

Linearity was studied by preparing 25, 50, 75, 100, 125 and 150% solutions from the standard stock solution of Piperine. Calibration curve was plotted by taking concentration on X-axis and peak area on Y-axis. Linearity was determined by the least square regression method. The slope value (m) and correlation coefficient (R^2) were calculated.

Precision (Repeatability)

Six piperine standard solutions of the same concentration were injected into the instrument and peak areas were interpreted to obtain peak areas. The concentrations and % RSD were estimated. The % RSD should be NMT 2%.

Accuracy

The accuracy of the method was studied by spiking a known amount of standard to the sample solution (50, 100 and 150%) and the % recovery was determined by using the optimized method. The percent difference between the expected and measured concentrations was determined and represented as % RSD. The % recovery and % RSD of the assay should be ± 100% and NMT 2% respectively.

Robustness

Robustness was studied by causing small variations in chromatographic conditions like flow rate, mobile phase ratio, pH, column, column temperature.

LOD and LOQ

LOD is defined as the least concentration of analyte that gives response accurately but need not be quantified exactly. LOQ is the least concentration of analyte that gives the accurate response and can be quantified exactly. These LOD and LOQ were calculated using the following formulae

$$LOD = 3.3\frac{\sigma}{s}$$

$$LOQ = 10\frac{\sigma}{s}$$

Where σ is the standard deviation of response
S is slope of the calibration curve

Assay Methodology

Assay of the marketed formulation was carried out by diluting the sample stock solution of tablet and syrup formulations to a suitable concentration and injected into the HPLC system. Peak areas were interpreted for the obtained peaks and the percent assay was calculated.

$$\%\ \text{Assay of Tablet} = \frac{AT \times WS \times DFS \times P \times T}{AS \times DFT \times WT \times 100 \times L} \times 100$$

AT = Peak area of the Test sample
AS = Peak area of Standard
WS = Weight of the Standard
WT = Weight of the Test sample
DFS = Dilution Factor of Standard solution
DFT = Dilution Factor of the Test sample
P = Purity of Standard
T = Average weight of Dosage form
L = Labelled claim

$$\%\ \text{Assay of Syrup} = \frac{AT \times WS \times DFS \times P \times T}{AS \times DFT \times VT \times 100 \times L} \times 100$$

AT = Peak area of Test sample
AS = Peak area of Standard
WS = Weight of Standard
VT = Volume of Test sample
DFS = Dilution Factor of Standard solution
DFT = Dilution Factor of Test sample
P = Purity of standard
T = Total volume of dosage form
L= Labelled claim

Degradation studies[16]

Forced degradation studies are performed to estimate the stability of the drug which in turn affects its purity, safety and potency. So, degradation studies are important to understand the stability of molecules under different stress conditions. The degradation limit defined by the regulatory agencies for validation of chromatographic assays is 5-20%.

Oxidation

20% hydrogen peroxide (H_2O_2) of 1 ml was added to 1 ml of standard stock solution of Piperine and the mixture was kept at 60°C for 30 min. The solution obtained was diluted to get (39ppm) solution. From this solution, 10 µl were injected and the obtained chromatograms were evaluated to assess its stability.

Acid and Alkali degradation Studies

1 ml of standard stock ssolution Piperine was taken separately in two different volumetric flasks (A, B). 1 ml of 2N Hydrochloric acid, 1ml of 2 N sodium hydroxide was added to A and B flask respectively. The above solutions were refluxed at 60°C for 30min. The obtained solutions were diluted to obtain (39ppm) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies

Piperine standard stock solution was kept in an oven at 105°C for 6 hr. 39ppm solution was prepared by diluting 1ml of obtained solution and 10µl volumes were injected into the system. The chromatograms were recorded to evaluate the Piperine stability.

Water Degradation Studies (Hydrolysis)

Stress testing was studied under neutral conditions by refluxing the drug in water for 6 hr at a temperature of 60°C. From the obtained solution, 1ml was taken and diluted to 39ppm solution and 10µl volumes were injected. The chromatograms were recorded to assess the stability.

RESULTS AND DISCUSSION

Optimization of mobile phase

The method development for assay of piperine was done by using Altima C_{18} column with mobile phase phosphate buffer (pH 3.5) and Acetonitrile in 40: 60 ratio but the peak obtained showed more retention time with USP pate count below the acceptance criteria. In the next trial BDS C8 column was used so that less retention time was obtained but USP plate count is not acceptable and the peak shape is not good. The optimized method with the following chromatographic conditions showed better results having less retention time, acceptable USP plate count with good peak shape and the chromatogram as shown in Figure 2.

Chromatographic conditions

Mobile phase	:	Buffer (0.1% OPA): ACN (50:50)
Diluent	:	Water: acetonitrile (50:50)
Flow rate	:	1 ml/min
Injection volume	:	10mL
Column	:	BDS C_8 150mm x 4.6mm x 5.0m.
Column temperature	:	30°C
Detector wave length	:	247nm
Run time	:	5 min
R_T	:	2.4 min

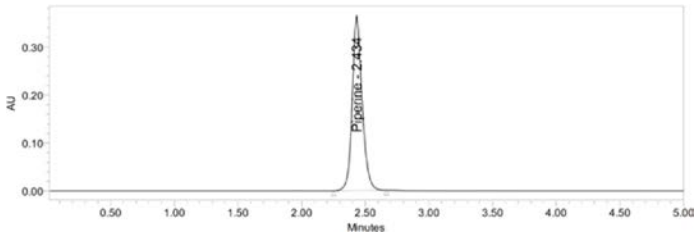


Figure 2: Chromatogram of piperine using optimum chromatographic conditions.

Solution stability

The stability of the prepared standard solution was checked for stability up to 24 hr and the solution was found to be stable.

Method validation

System suitability parameters

The optimized method showed USP plate count of more than 2000 with tailing factor less than 1.5. The % RSD for peak area and retention time was found be less than 2 which indicate that the developed method was suitable for the estimation of Piperine. The results of the System suitability study was given in Table 1.

Specificity

Blank and placebo were injected separately into HPLC. The chromatograms obtained didn't show any peaks at the R*t* of piperine.

Linearity

A standard curve was plotted by taking 25, 50, 75, 100, 125 and 150% solutions from the standard stock solution of piperine. The correlation coefficient (R^2) for the linearity plot was found to 0.999 (Figure 3) which confirms that the chosen concentration range of piperine follows linearity. The results of linearity were given in Table 2.

Precision (Repeatability)

The repeatability of the method was studied by interpreting peak areas of the samples injected

in different days (Table 3). % RSD was calculated and found to be 0.16 which confirms that the developed method is precise.

Accuracy

The % recovery was calculated for the 50, 100, 150% spiked solutions and the average % recovery was found to be 98.97. The % RSD was found to be 0.73 which indicates that the accuracy of the method was within the acceptable range. The recovery results were shown in Table 4.

Table 1: System suitability studies for optimized method.

Sample	Piperine
Retention time	2.4 min
USP plate count	4607
Peak area	1964775
Tailing factor	1.44

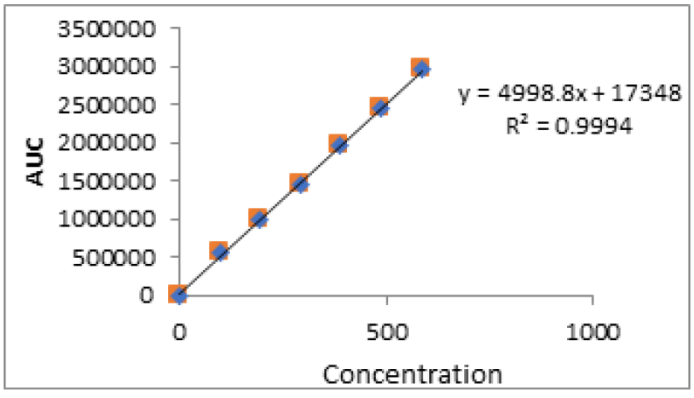


Figure 3: calibration curve for linearity.

Table 2: Results for Linearity.

Y-intercept	17348
Slope	4998
Correlation coefficient (r^2)	0.999
Regression equation	y = 4998.x + 17348
Linearity range	25-150%

Table 3: Results of precision.

Sl.No.	RT	Peak area
1	2.431	1970634
2	2.434	1968746
3	2.435	1965907
4	2.436	1973514
5	2.436	1965721
6	2.437	1970924
	MEAN	1969241
	% RSD	0.155

Table 4: Results of recovery studies.

Spiked level	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery
50%	19.5	19.27	98.80
50%	19.5	19.61	100.57
50%	19.5	19.41	99.53
100%	39.0	38.25	98.09
100%	39.0	38.34	98.31
100%	39.0	38.50	98.72
150%	58.5	57.91	98.99
150%	58.5	57.88	98.93
150%	58.5	57.99	99.14
Mean		99.01	
SD		0.73	
% RSD		0.73	

Robustness

Robustness was studied by changing flow rate (0.8-1.2 ml/min), mobile phase ratio (55:45 and 45: 55), column temperature (28, 32°C). These changes didn't show much influence on R_t and peak area. The % RSD for the obtained results was found to be within the limits and was shown in Table 5.

LOD and LOQ

LOD and LOQ of piperine for the developed method were found to be 0.076 and 0.230 **µg/ml** respectively.

Assay

The developed method was successfully employed for the assay of piperine in tablet formulation. The % assay of piperine for tablet and

Table 5: Results of Robustness.

Parameter	Variation	%RSD
Flow rate	0.9ml/min	0.134
	1.1ml/min	0.405
Temperature	28°C	0.719
	32°C	0.802
Mobile phase	45:55	0.008
	55:45	0.289

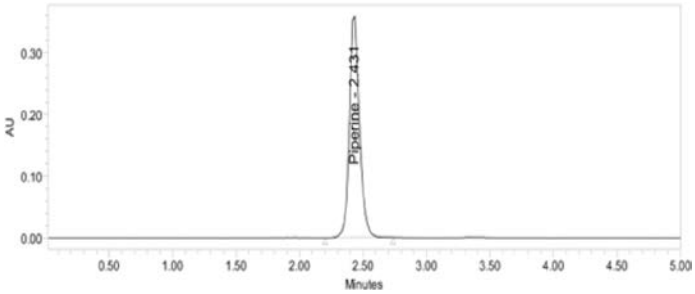


Figure 4: Chromatogram of sample (tablet).

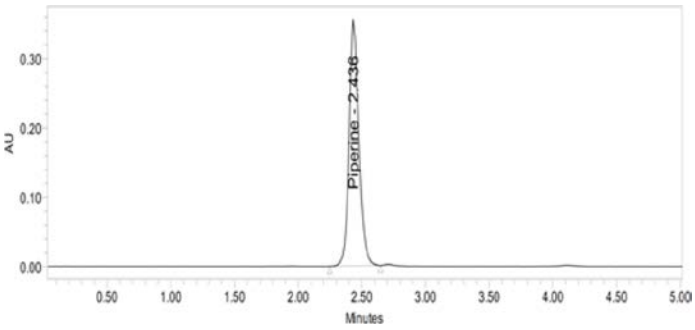


Figure 5: Chromatogram of sample (syrup).

syrup formulation was found to be 95.216 and 95.158 respectively and were shown in Figure 4 and Figure 5.

Degradation studies

In order to assess the intrinsic stability of the piperine in the dosage form forced degradation studies were performed. The stressed sample did not show any interfering peaks of degradation product which indicates that the developed method is stable and the results are shown in Table 6.

DISCUSSION

A stable RP-HPLC method was developed for assay of piperine in tablet dosage form and the same method was successfully employed for assay of piperine in syrup. The chromatographic conditions like BDS C8 column with mobile phase composition of 0.1% OPA buffer: Acetonitrile in 50:50 ratio at 1.0 ml/min flow rate, using PDA detector at λ of 247 nm eluted piperine at R_T of 2.4 min. The % assay of piperine for tablet and syrup formulation was found to be 95.216 and 95.158 respectively. For forced degradation studies peak purity angle should be less than the peak purity threshold according to ICH guidelines,[17] and the same was obtained in the stability studies which indicates that the developed method is stable.

Table 6: Results of Degradation studies.

Sl. No	Name of degradation	RT (min)	Peak area	Purity angle	Purity threshold	% Degradation
1	Acid degradation	2. 437	18 78132	0.109	0.314	4.51
2	Base degradation	2.438	1912590	0.184	0.373	2.75
3	Peroxide degradation	2. 435	1929138	0.098	0.295	1.91
4	Thermal degradation	2. 433	1949944	0.098	0.299	0.85
5	Water degradation	2. 431	1949139	0.099	0.293	0.90

CONCLUSION

In the present world, a rapid, economic and stable analytical method was developed for the assay of Piperine in syrup and tablet dosage forms by RP-HPLC. All the validation parameters were tested according to the ICH guidelines and the results fall within acceptable limits. The developed method was found to be specific for the analyte of interest in presence of excipients. As the retention time is short, the analyst can analyze more number of samples in less duration. So, the proposed method can be successfully employed for routine analysis of Piperine in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Acharya Nagarjuna University, Nirmala College of Pharmacy for their support to carry out the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

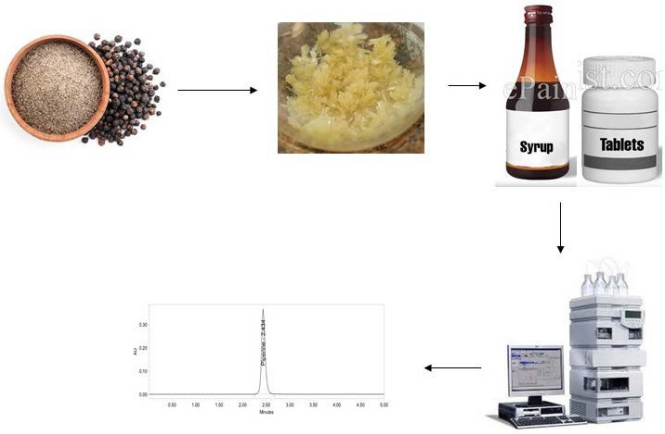
RP-HPLC: Reverse phase High Performance Liquid Chromatography; **ACN:** Acetonitrile; **RSD:** Relative Standard Deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification.

REFERENCES

1. Suvarna Y, Abdul Rahaman SK. Pharmacotherapeutic properties of black pepper: A systematic review. Asian J Res Chem Pharm Sci. 2019;7:293-304.
2. Buckingham J (ed.). Dictionary entries. In: Dictionary of alkaloids. 2nd ed. New York: CRC press Press; 2008;475-67.
3. Amar S, Amar D. Piperine: A bioenhancer. Int J Pharm Res Technol. 2019;1(1):01-5. doi: 10.31838/ijprt/01.01.01.
4. [cited 30/3/2022] Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Piperine#section=Names-and-Identifiers.
5. Shivani M, Spandana K, Himabindhu J, Ramanjaneyulu K. Evaluation of *in-vitro* antiurolithiatic activity of *Piper nigrum*. Int. J Pharm. Sci Med. 2018;3(8):43-50.
6. Suvarna Y, Rahaman SKA. *In vitro – in vivo* Evaluation of Antiurolithiatic activity of piperine from *Piper nigrum*. Res J Pharm Technol. 2020;13(1):63-8. doi: 10.5958/0974-360X.2020.00011.6.
7. Vipul U, Neeru S, Joshi Himanshu M, Amreesh M, Manoj M, Singh BP, *et al.* Development and validation of rapid RPHPLC method for estimation of piperine in *Piper nigrum* L. Int J Herb Med. 2013;1(4):6-9.
8. Santosh Linn MK, Shaila D, Rajyalakshmi I, Sanjeeva Rao I. RP - HPLC method for determination of piperine from *Piper longum* Linn. and *Piper nigrum*. E-journal of chemistry. 2005;2(2):131-5.
9. Khismatrao A, Bhairy S, Hirlekar R. Development and validation of RP-HPLC method for simultaneous estimation of curcumin and piperine. Int J App

Pharm. 2018;10(5):43-8. doi: 10.22159/ijap.2018v10i5.21140.
10. Narasimhaji CV, Murugammal S, Kusuma G, Meena AK. Ravindra Singh Shrikanth N, Hazra J. Isolation of piperine from a poly herbal formulation and its quantitative evaluation. J Acupun Tradit. Med. 2019;3(1):1-6.
11. Vyas J, Itankar P, Tauqeer M, Kelkar A, Agrawal M. Development of HPTLC method for estimation of piperine, guggulsterone E and Z in polyherbal formulation. Pharmacogn J. 2013;5(6):259-64. doi: 10.1016/j.phcgj.2013.10.003.
12. Shanmugasundaram P, Maheswari R, Vijayaanandhi M. Quantitative estimation of piperine in herbal cough syrup by HPTLC method. Rasayan J Chem. 2008;1(2):212-7.
13. Parameswaran S. Quantitative estimation of piperine and diosgenin in ayurvedic formulation. Br J Pharm Res. 2014;4(8):910-9. doi: 10.9734/BJPR/2014/8310.
14. Suvarna Y, Abdul Rahaman SK. Formulation, evaluation and study of antiurolithiatic activity of piperine tablets. Int J Biol Pharm Allied Sci. 2021;10(10):3629-45.
15. Suvarna Y, Abdul Rahaman SK. Formulation, evaluation and study of antiurolithiatic activity of piperine syrup. Drug Invent Today. 2020;13(2):163-7.
16. Sowmyalakshmi Venkataraman MM. Forced degradation studies: Regulatory guidance, characterization of drugs and their degradation products- A review. Drug Invent Today. 2018;10(2):137-46.
17. Naazneen S, Sridevi A. Development of assay method and forced degradation study of valsartan and sacubitril by RP-HPLC in tablet formulation. Int J Appl Pharm. 2017;9(1):9-15. doi: 10.22159/ijap.2017v9i1.15448.

GRAPHICAL ABSTRACT



SUMMARY

A stable RP-HPLC method was developed for assay of piperine in tablet dosage form and the same method was successfully employed for assay of piperine in syrup. The chromatographic conditions like BDS C8 column with mobile phase composition of 0.1% OPA buffer: Acetonitrile in the ratio of 50:50 at flow rate of 1.0ml/min, detection wavelength of 247nm using PDA detector eluted piperine at RT of 2.4 min. The % assay of piperine for tablet and syrup formulation was found to be 95.216 and 95.158 respectively. Forced degradation studies were performed to assess the intrinsic stability of the drug in its dosage form. The stressed sample did not show any interfering peaks of degradation product which indicates that the developed method is stable.

Cite this article: Suvarna Y, Rahaman SKA, Madhavi A. Novel Stability Indicating Method for Determination of Major Alkaloid in Black Pepper by RP-HPLC in Different Pharmaceutical Dosage Forms. Pharmacog Res. 2022;14(2):219-24.