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Development and Validation of Rapid RP-HPLC Method for Estimation of Piperine in *Piper nigrum* L.

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ABSTRACT

A simple, economic, rapid, precise reverse phase high pressure liquid chromatographic method has been developed and assay was validated for the determination of Piperine in *Piper nigrum* extract. Piperine, an alkaloid, it is helpful in reducing inflammation, improving digestion, and relieving pain and asthma. It is reported to improve the production of serotonin, it may relieve stomach ulcerations. It improves the bioavailability of other nutritive substances including beta carotene, curcumin, selenium, pyroxidine, glucose, and amino acids. This method was carried out by using (250x4 mm, 5 µ) C18 column with a mobile phase consisting Acetonitrile, Water, Acetic acid (60:39.5:0.5). The flow rate was set to 1.0 ml/min with UV detection at 340 nm with run time 10 min and injection volume set at 20 µl. The percentage of RSD for precision and accuracy of the method was found to be less than 2%.The percentage recovery of Piperine was found to be 99.29% and the developed method was validated in terms accuracy, precision, robustness and recovery. This selective method is found to be accurate, repeatability and effectively used for the Piper extract in marketed sample with better chromatographic conditions.

Keywords: Piperine, RP HPLC method, UV Detection, validation, C18 column.

1. Introduction

The Piperine (1-piperoylpiperidine), a nitrogenous pungent substance, is an alkaloid presents in the fruits of black pepper (*Piper nigrum*) and other piper species (family: Piperaceae) [1]. Piperine, an alkaloid, is found in Piper longum obtained from botanical sources is about 98% pure [1, 2]. Since piperine has been recognized as a main alkaloid in these plants. Piperine (PIPE) is a natural alkaloid which is used as Bio enhancer and responsible for the pungency of black pepper and long pepper, along with chavicine (an isomer of piperine) [2]. It has also been used in some forms of traditional medicine and as an insecticide [3]. Recent pharmacological studies have shown that piperine possess anti-inflammatory and analgesic effect, anticonvulsant, anti-ulcer, anti-depressant effect, cytoprotective effect and antioxidant activity [4]. Black pepper (*piper nigrum*) is a widely used hot spice [1]. Furthermore it is used in the traditional medicine Piperine (Fig. 1) is the main compound leading to bioactivity of black and white pepper. Its pungency has been estimated as 100000-200000 Scoville Unit [5]. Piperine is an alkaloid and it is the carboxamide of Piperic acid and Piperidine [5]. It shows low solubility in water, but ethanol and other organic solvent are suitable for dissolving this substance [6]. In recent decades, Piperine came into the focus of pharmaceutical research. It has antibacterial, antioxidant, anti-inflammatory, antiarthritic and other effects [7]. The most interesting point is that Piperine increases the bioavailability of a number of therapeutic drugs as well as phytochemicals [8]. The phytoconstituents of P. nigrum and P. longum fruits include volatile oil, other minor alkaloids such as piplartin, piperlogumine, piperidine, starch, resin and piperine which is pungent alkaloid is the main therapeutically active constituent of this plant [9, 10].

2. Experimental

Standard of piperine purchased from Natural Remedies. HPLC grade water, Acetonitrile (ACN), Methanol (MeOH), Acetic acid was purchased from Renkem (Mumbai, India).

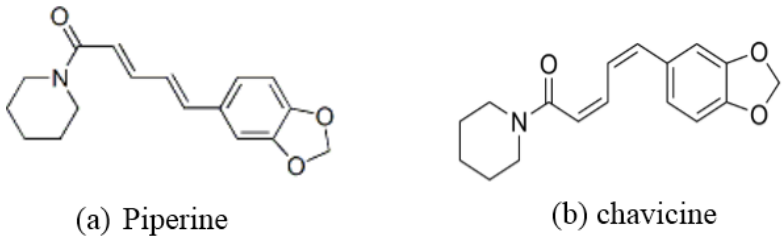

Fig 1: Structure of piperine & chavicine

Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with this mobile phase, Acetonitrile: Water: Acetic acid (60:39.5:0.5). The high percentage of recovery of Piperine was found to be 99.29% indicating that the proposed method is highly accurate, applied for the determination of Piperine. System suitability parameters and Chromatographic Conditions of Piperine were given in Table 1.

Table 1: Chromatographic conditions of Piperine

S. No.	Test Conditions	Result
1	Elution	Isocratic
2	Wave Length	340 nm
3	Mobile Phase	Acetonitrile: Water: Acetic acid (60:39.5:0.5)
4	Column	C18
5	Retention Time	5.6
6	Flow	1 ml/min
7	Runtime	10 min

2.1 Chemicals and Reagents

The Piper extract purchased from the Unicorn Natural Products Limited. Other reagents used like Methanol, Acetonitrile, Water and Acetic acid were purchased from Renkem (Mumbai, India).

2.2 Analytical conditions

HPLC (Shimadzu, LC 2010A, Japan), Autosampler, UV-Detector was used for the analysis of Piperine. The data was acquired on the LC solution administrator data system (Japan) and Licrocart C_{18} column (250 mm X 4.6 mm, 5 µm) (California, USA) was used. Injections were carried out using a 20 µl loop at room temperature and the flow rate was 1 ml/min. Detection was performed at 340 nm with 10 min runtime and mobile phase (Acetonitrile: Water: Acetic acid (60:39.5:0.5)) was filtered through 0.45 µm Millipore filter and degassed by sonication for 30 min.

2.3 Instrumental Apparatus

The development and validation of the assay was performed on HPLC (Shimadzu, LC 2010A, Japan), Autosampler, UV-Detector. Analytical column Licrocart C18. 250×4.6mm, with 20 µL loop, LC solution software was used. Detection was performed at 340 nm using UV detector.

2.4 Preparation of Stock & and Sample Solutions

A 10 mg amount of Piperine standard was weighed accurately and dissolved in 10 ml methanol in a 10 ml volumetric flask to get 1000 ppm. From the standard solution we prepared required concentration of 100 ppm on serial dilutions. 10 mg of Piperine sample was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 50 ml methanol were added and the solution was sonicated for 30 min. The flask was filled upto 100 ml with methanol then mixed and filter.

2.5 Calibration Curve

Seven different concentrations of stock solution (40, 50, 60, 70, 80, 90,100 ppm) after dilution with methanol were injected in triplicates and regression equation and co-efficient of correlation (r^2) was derived (Table 2).

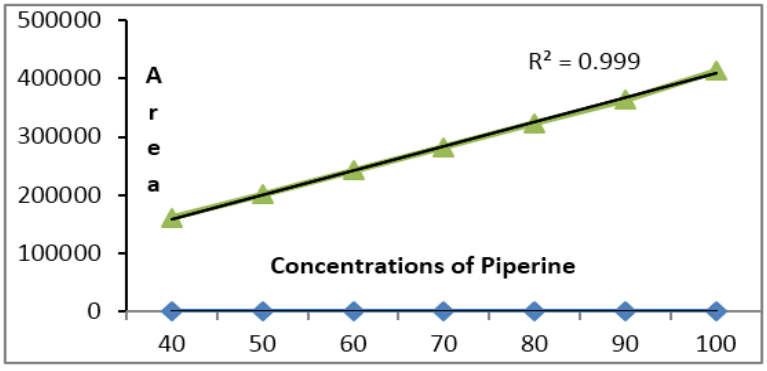

Fig 2: Calibration curve of piperine

2.6 Validation of method

2.6.1 Range of linearity:

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 40, 50, 60, 70, 80, 90,100 ppm for Piperine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 41488x + 11828. Linearity values can show in Table 2 and the calibration curve is given in Fig. 2.

Table 2: Validation parameters of the developed HPLC method quantification of Piperine

S. No.	Validation Parameters	Results
1	Linearity range (ppm)	40-100 ppm
2	Correlation coefficient (r2)	0.999
3	Regression equation	y = 41488x +11828
4	LOD (ppm)	5.0 ppm
5	LOQ (ppm)	5.0 ppm
6	Method precision (RSD %)	0.73
7	Intermediate precision (RSD %)	
7.1	Interday (%)	0.89
7.2	Intraday (%)	1.30
8	RSD % (Linearity of the method)	1.32

2.6.2 Limits of detection (LOD) and quantification (LOQ):

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. The LOD value is 5 ppm. The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ observed is 5 ppm and the values are given in Table 2.

2.6.3 Accuracy (recovery):

Accuracy of the method was ascertained by spiking the pre-analysed samples with known amount of standard solution (50%, 100%, and 150%). The average percentage recovery was estimated by applying values of peak area to the regression equations of the calibration graph. Three replicate samples of each concentration level were prepared.

Recovery test was performed at 3 different concentrations i.e. 40 ppm, 60 ppm, 80 ppm. Results are given in Table 3.

Table 3: Recovery Percentages of Piperine

Recovery	Conc. of sample	Recovery	% of recovery
50%	40 ppm	42.52	100.01
100%	60 ppm	62.99	99.29
150 %	80 ppm	80.63	98.99

2.6.4 Method precision (repeatability):

The precision of the instruments was checked by repeatedly injecting and analyzing (n=6) standard solution 70 ppm. The results are reported in terms of relative standard deviation (RSD). The interday and intraday precision of the proposed method were determined by analyzing standard solution at different concentrations (40, 50, 60, 70, 80, 90,100 ppm) three times on the same day and on three different days. The results are reported in terms of RSD.

3. Results and Discussion

3.1 Optimization of the chromatographic conditions

The total run time of Piperine was found to be 10 minutes and the Piperine appeared on chromatogram at 5.668 Piper extract (Fig. 3). The retention time of reference standard (Piperine) was observed to be 5.655 minutes (Fig. 4). This indicates that the present HPLC method is rapid; easy and convenient. When the same drug solution was injected 6 times, the retention time of the peak was found to be same. The Sample nature, its solubility and molecular weight confirms the correct selection of the stationary phase. The drug Piperine being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. The elution of the compound from the column was influenced by polar mobile phase. The concentration of the acetonitrile and water were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained and the results of Extract of Piperine drug is given in Table 4.

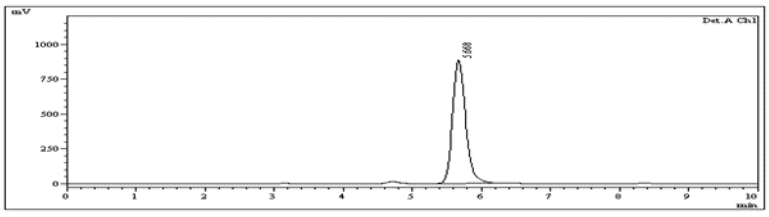

Fig 3: Chromatogram of Piper extract

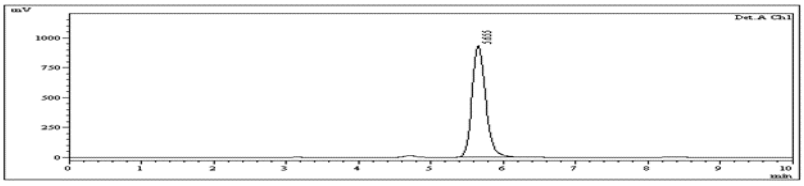

Fig 4: Chromatogram of Reference standard (Piperine)

3.2 HPLC analysis of Piperine in *Piper nigrum* Extract

Quantitative estimation of Piperine in *Piper nigrum extract* given in Table 4. It was 95.61% w/w in *Piper nigrum* extract.

Table 4: Piperine content in extract of *Piper nigrum L.*

Name of Extract	Piperine content (%)
Piper nigrum	95.61%

4. Conclusion

The assay of Piperine in *Piper nigrum* extract is very accurate, simple and rapid. This method was isocratic and the mobile phase do not contain any buffer and validated for the determination of purity and assay of Piperine. The developed method is sensitive, simple, and accurate, and can be employed for monitoring the purity of Piperine in Piper extract. The method has been proved to be selective and stable.

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