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RP - HPLC Method for Determination of Piperine from *Piper longum* Linn. and *Piper nigrum* Linn

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Abstract: *Piper longum* Linn. and *Piper nigrum* Linn. are used as spices and medicines. Quantitative determination of piperine was undertaken to provide an easy and simple analytical method, which can be used as a routine quality control method. RP-HPLC was performed using methanol and water as mobile phase. The detection and quantification was performed at a wavelength of 345nm. Linearity of detector response for piperine was between the concentrations 0.005% to 0.1%. The correlation coefficient obtained for the linearity was 0.998. The assay value of piperine for fruit and root of *P. longum* was found to be 0.879% and 0.31%. The assay value of piperine for fruit of *P. nigrum* was 4.5%. The recovery value of standard piperine was 99.4%. Low value of standard deviation and coefficient of variation are indicative of high precision of the method.

Key words: *Piper longum*, *Piper nigrum*, Piperine, HPLC.

Introduction

Fruits of *Piper longum* Linn. and *Piper nigrum* Linn. (Piperaceae) are used as spices and medicines. Roots of *P. longum* Linn. are used in traditional alternative Indian System of Medicine. Both the plants contain piperine, a principle pungent alkaloid¹. The major constituent piperine possesses central nervous system (CNS) depressant, antipyretic, analgesic, anti-inflammatory, antioxidant and hepatoprotective properties²⁻⁴. In humans, piperine increases the bioavailability of antitubercular drugs when given together¹. A few methods have been reported for the estimation of piperine by UV spectrophotometry⁵, TLC-UV densitometry⁶, HPTLC⁷ and HPLC⁸ techniques.

As a part of our efforts to develop a simple gradient RP-HPLC method using methanol and water as a mobile phase for direct determination of piperine in *P. longum* Linn. and *P. nigrum* Linn., to investigate the distribution of this compound in different plant parts. The proposed method can be used for routine quality control study of raw drugs and finished products, which contain piperine as a marker compound.

Materials and Methods

Fruits and roots of *P. longum* Linn. and fruits of *P. nigrum* Linn. were procured from the local market of Hyderabad, Andhra Pradesh. Standard piperine was procured from M/s Sigma –Aldrich Chemie GmbH, Germany.

Sample preparation

The standard piperine solution was prepared by dissolving 5mg of piperine in 5ml of absolute alcohol and it was diluted to obtain 0.005%, 0.01%, 0.02% and 0.1% concentrations. The 4% alcoholic extracts of fruit and root of *P. longum* and 0.8% alcoholic extract of fruit of *P. nigrum* were prepared by soaking the respective material for 18h in absolute alcohol. The extract was centrifuged at 3000rpm and then filtered through 0.2µ membrane filter using high-pressure vacuum pump and the clear solutions were used for HPLC fingerprint analysis.

Instrumentation

A gradient HPLC (Shimadzu HPLC Class VP series) with two LC- 10 AT VP pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and reverse phase Luna 5µ C₁₈ (2) Phenomenex column (250mm X 4.6mm) was used. The HPLC system was equipped with Class VP series version 6.1 software (Shimadzu). The mobile phase components methanol: water were filtered through 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 220 – 240 kgf / cm². The column temperature was maintained at 27°C. 20µl of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton).

Results and Discussion

Standard piperine solutions of 0.005%, 0.01%, 0.02% and 0.1% concentration were analyzed for studying the linearity and area count obtained for these solutions are presented in **Table-1**. Piperine showed good linearity in the concentration range of 0.005% - 0.1% with a correlation coefficient of 0.998. The precision of the method was also studied by injecting a single sample solution five times (**Table-2**) and finding out the standard deviation and coefficient of variation. The standard deviation and coefficient of variation were found to be 0.161 and 0.219.

Table 1. Area counts for standard piperine

S. No.	Concentration of piperine (%)	Area counts
1	0.005	3752832
2	0.01	7328721
3	0.02	14495677
4	0.1	67668536

The HPLC chromatogram of standard piperine at an optimum wavelength of 345nm showed a mean area (**Table-2**) of 7328514.2 at a retention time of 23.733min (**Figure-1**). The recovery value of standard piperine was 99.4%. The HPLC chromatogram of fruit of *Piper longum* corresponding to standard piperine was shown at a retention time of 23.616 min with an area of 25754634 at a wavelength of 345nm (**Figure – 2**). The HPLC chromatogram of root of *Piper longum* corresponding to standard piperine was shown at a retention time of 23.723min with an area of 9101266 at a wavelength of 345nm (**Figure – 3**). The HPLC chromatogram of fruit of *Piper nigrum* corresponding to standard piperine was shown at a retention time of 23.467min with an area of 26230008 at a wavelength of 345nm (**Figure-4**). The variation in retention time of peak of piperine in chromatograms

of *P. longum* and *P. nigrum* may be due to the presence of other chemical constituents. The quantitative evaluation of piperine in fruit and root of *P. longum* was 0.879% and 0.31%. The quantitative evaluation of piperine in fruit of *P. nigrum* was 4.5%.

Table 2. Determination of standard deviation and coefficient of variation for standard piperine

S. No.	Concentration of piperine (%)	Area
1	0.01	7328721
2	0.01	7329368
3	0.01	7330168
4	0.01	7325643
5	0.01	7328671

Figure 1. HPLC chromatogram of standard piperine

Figure 2. HPLC chromatogram of fruit of *Piper longum* Linn.

Figure 3. HPLC chromatogram of root of *Piper longum* Linn. **Figure 4.** HPLC chromatogram of fruit of *Piper nigrum* Linn.

The piperine content in fruit of *P. nigrum* was reported to be 50.78mg/g of pepper⁷ and 2-5%⁸. The piperine content in fruits of *P. nigrum* and *P. longum* was reported to be 3-6% and 0.6-1.6% by HPLC technique⁹. The proposed method can be used to standardize *Piper species* on the basis of piperine as a marker compound.

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