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

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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 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.

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 pKₐ value is 13.2,³ which indicates that it is very weak acid. Chemically it is 1-piperoylpiperidine (or) (2-{(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.³⁻⁴ Several methods are available for estimation of Piperine in its extract,⁵⁻⁹ 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 Thermo Fisher 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 C₈ (150mm x 4.6mm x 5.0 μm) 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 formulation¹⁴

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...
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.
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 solution 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 30 min. 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 C18 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 plate 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 C8 150mm x 4.6mm x 5.0m.
  - Column temperature: 30°C
  - Detector wave length: 247nm
  - Run time: 5 min
  - $R_t$: 2.4 min

**Degradation studies**

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 (H₂O₂) 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.
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 to 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 RT 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 (r²) 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>
<thead>
<tr>
<th>Sample</th>
<th>Piperine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>2.4 min</td>
</tr>
<tr>
<td>USP plate count</td>
<td>4607</td>
</tr>
<tr>
<td>Peak area</td>
<td>1964775</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>RT</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.431</td>
<td>1970634</td>
</tr>
<tr>
<td>2</td>
<td>2.434</td>
<td>1968746</td>
</tr>
<tr>
<td>3</td>
<td>2.435</td>
<td>1965907</td>
</tr>
<tr>
<td>4</td>
<td>2.436</td>
<td>1973514</td>
</tr>
<tr>
<td>5</td>
<td>2.436</td>
<td>1965721</td>
</tr>
<tr>
<td>6</td>
<td>2.437</td>
<td>1970924</td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td>1969241</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>0.155</td>
</tr>
</tbody>
</table>

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 RT and peak area. The % RSD for the obtained results was found to be within the limits and was shown in Table 5.

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

Figure 3: calibration curve for linearity.

Figure 3: calibration curve for linearity.
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.

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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

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 1.0 ml/min, detection wavelength of 247 nm 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.