# QUANTITATIVE ANALYSIS OF PIPERINE IN AN AYURVEDIC FORMULATION USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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**Abstract:**

A simple, rapid and precise reverse-phase high performance liquid chromatographic method has been developed for the quantitative determination of piperine in an Ayurvedic polyherbal formulation: Ajmodadi churna. Chromatographic analysis was carried out on cosmosil $C_{18}$ column (150mm x 4.6mm, 5µm particle) with a mobile phase of methanol: water in the ratio of 65:35 at a flow rate of 1.0 mL $min^{-1}$. Quantitation was performed using a PDA-detector at 344 nm. Linear response for piperine was obtained over a range of 200 to 4800 ng $mL^{-1}$. The method was validated for linearity, precision, accuracy and can be effectively used to evaluate quality of Ajmodadi churna.

**Keywords: RP-HPLC, Piperine, Ajmodadi churna, polyherbal formulation.**

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## Introduction:

Ayurveda is practiced widely in India, Srilanka and other countries **[1]** and Ayurvedic preparations are either of herbal origin, mineral origin, animal origin or combination of them. The safety and efficacy of these formulations is closely correlated with the quality and the source of raw materials used in their production **[2]**. It is generally possible to estimate a phytochemical marker for the ingredient plant raw material using various analytical techniques like TLC, HPLC and HPTLC, using these techniques it is possible to detect the presence of the marker phytochemical compound and quantify it to ascertain the limits in the final formulation **[3, 4]**.

The Ajmodadi churna (AC) is well known Ayurvedic formulation official in Ayurvedic Formulary of India, traditionally used for abdominal pain, carminative, antispasmodic and helps in all painful conditions like sciatica and stiffness **[5]**. As the literature survey there is no proper analytical method available for the quantitative estimation of piperine in Ajmodadi churna. The present study focused on to develop a rapid, efficient and reproducible method for the analysis of piperine in Ajmodadi churna by HPLC.

## Materials and Methods:

All the chemicals used were of HPLC grade (CDH Chemicals Ltd.). Piperine standard was procured from Sigma Chemicals. Ajmodadi churna was procured from the local market**.**

## Standard:

Stock solution of Piperine (1000 ppm) was prepared in methanol using amber colored volumetric flasks. Further standard solutions of 100ppm, 10ppm, 1ppm were prepared from standard stock solution by dilution with mobile phase containing methanol and water. These standards were stored at $40^{o}C$ protected from light and brought to room temperature before use.

**Sample Preparation**:

Powdered Ajmodadi churna was refluxed with 60 ml Methanol then filtered the extract and rerefluxed the marc left with 40 ml of Methanol for 1 hour. After the filtration filtrates were combined. Concentrated the methanolic extract under vacuum till the semisolid mass was obtained. The residue was dissolved in 75 ml Methanol and filtered through sintered glass funnel by vacuum filtration. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100 ml volumetric flask and volume was made up with Methanol **[6, 7].**

**Instrumentation and Chromatographic Conditions: [8-9]**

Chromatography was performed with Jasco's Binary Type High Performance Liquid Chromatography; comprising two PU-1550 pumps with 20µl loop and a Jasco multi wavelength detector MD-1510. A Cosmosil $C_{18}$ column (150mm x 4.6mm, 5µm particle) was used for the analysis. The mobile phase was a mixture of Methanol: water 65:35 (v\v) delivered at a flow rate of 1.0 mL $min^{-1}$ .The data was collected at wavelength of 344 nm. Peak of piperine was identified by comparison with retention time of standard piperine.

**Method Validation:**

***System Suitability:***

System suitability was determined by injecting, working standard solution of piperine (1000 ng/ml) five times. The peak area values and the retention time of piperine were noted for each applied concentration of piperine. The coefficient of variation for the peak area and retention times was calculated.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):**

Limit of quantitation (LOQ) was established at a signal-to-noise ratio of 1:10 and Limit of detection (LOD) was established at a signal-to noise ratio of 1:3.

**Linearity:**

Linearity was evaluated by analysis of working standard solutions of piperine of different concentrations. The peak area and concentration of piperine was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The response was linear in the range of 200 to 4800 ng $mL^{-1}$ **(Table 1)**.

**Precision:**

The precision for the method was analysed by determining intra-assay precision and intermediate precision. The Intra-Assay/within day precision was carried out on one day at three different concentration levels i.e. 250 ng/ml, 1600 ng/ml , 2500 ng/ml , with three replications of each. The Inter-day precision was carried out on multiple days. The experiment carried out for intraday precision was repeated in the same manner on two more days by analyzing in triplicate. The method was found to be precise **(Table 1).**

**Assay:**

Standard and sample solutions were injected in HPLC system. The amount of piperine present per gram or per ml of formulation was calculated by comparison of the areas measured for the sample with the calibration curve constructed from peak area obtained from standard solution of piperine. The percent content of piperine was found to be 1.74% in the formulation **(Table 1)**.

**Accuracy:**

To check the accuracy of the developed methods and to study the interference of formulation excipients, recovery experiment was carried out by standard addition method. A known amount of sample was taken in different tubes for three different concentration levels. To each tube known amount of piperine was added. Each sample was extracted and

analysed by the developed HPLC method in replicates and the amount of piperine recovered for each level, was calculated.

**Result and Discussion:**

Piperine has been quantified by some researcher **[10]**. But there are no reported methods for quantitation of piperine from Ayurvedic polyherbal formulations like Ajmodadi churna by reverse phase HPLC method. The current research paper involves quantitation of piperine from Ajmodadi churna which has black pepper as one of the ingredients. Piperine standard was detected and accurately quantified by using RP-HPLC with Methanol: Water, 65:35 (v/v) as mobile phase **(Fig-1)**. The identity of the piperine in the formulation was confirmed by the comparing the retention time of chromatogram obtained from the sample with that obtained from the piperine standard **(Fig-2)**. The range of linearity was found to be from 200 to 4800 ng $mL^{-1}$. The results show that within the concentration range there is an excellent correlation between peak area and concentration of piperine. The correlation coefficient was found to be 0.9997. The concentration of piperine in formulation was found to be 1.74%. System suitability, intra-day assay precision and inter-day assay precision were measured. RSD values were less than 2%, indicating that developed method is precise and reproducible **(Table-1)**. The value of mean % recovery was found to be 99.18 % which indicates good accuracy of method **(Table-1)**.

**Conclusion:**

The reported RP - HPLC method for quantitation of piperine in Ajmodadi churna was found to be sensitive, simple, fast and reliable for routine quality control analysis of Ajmodadi churna. Thus the developed method can be applied for the quantitation of piperine to various polyherbal formulations along with Ajmodadi churna.

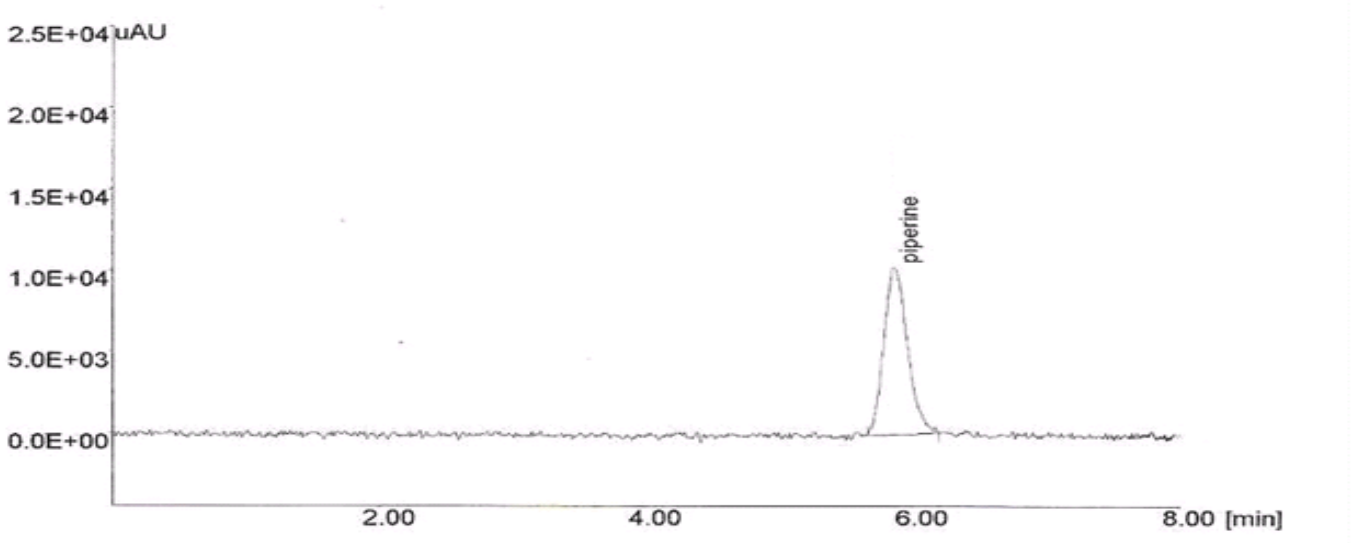


**Figure 1. Chromatogram of Piperine standard**

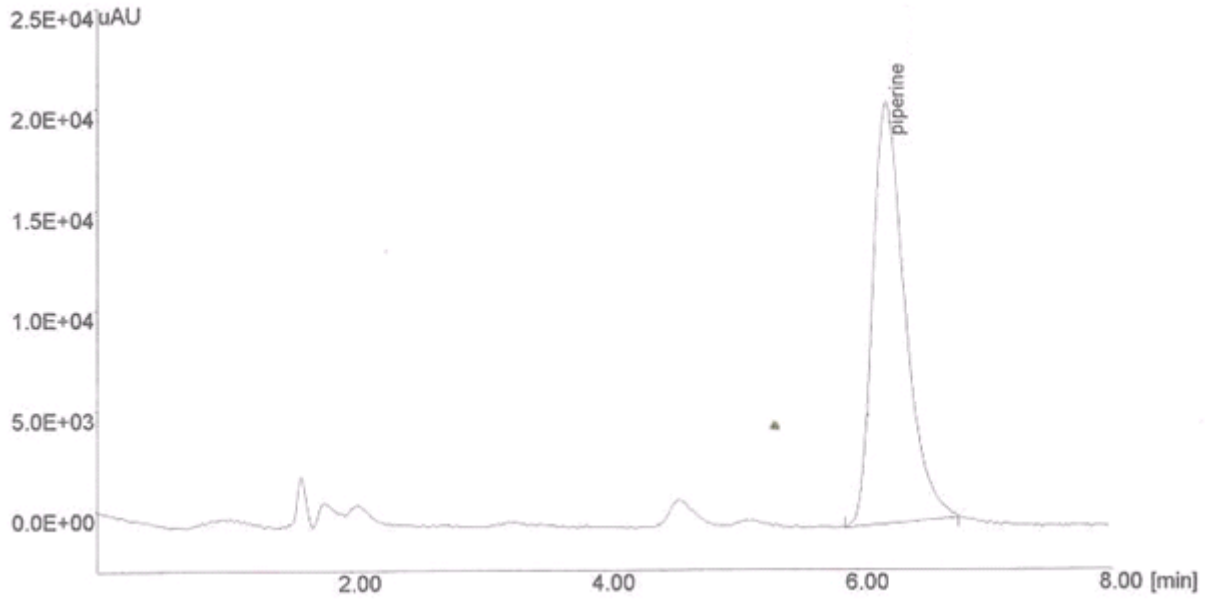


**Figure 2. Chromatogram of Piperine in sample**

**Table 1. Result of validation parameters:**

| **Parameters** | | **Range** |
|---|---|---|
| LOD (ng) | | 35.23 |
| LOQ (ng) | | 100.66 |
| Linearity range (ng) | | 200–4800 |
| Correlation coefficient | | 0.9997 |
| Slope | | 3.344 |
| Intercept | | 1006 |
| Retention Time | | 6.19 |
| Assay (% content) | | 1.74 |
| Recovery (Mean %) | | 99.18 |
| Precision (RSD %) | Inter-day (n = 3) | 1.15–1.43 |
| | Intra-day (n = 3) | 1.15–1.23 |

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