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QUANTITATIVE ANALYSIS OF PIPERINE FROM AYURVEDIC POLYHERBAL FORMULATIONS 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 from Ayurvedic Polyherbal formulations Arkavati, Krvyadras and Marichyadi taila. 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 volume ratio of 70:30 at a flow rate of 1.0 mL min^{-1}. Quantitation was performed using a PDA-detector at 342 nm. Linear response for piperine was obtained over a range of 200 to 3000 ng mL^{-1}. The method was validated for linearity, precision, accuracy and can be effectively used to evaluate quality of Arkavati, Krvyadras and Marichyadi taila.

KEYWORDS

RP-HPLC**,** piperine, Arkavati, Krvyadras, Marichyadi taila

1. 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].

In Ayurveda there are several different types of formulation like Vatis-Gutika (Pills), Rasa yoga (mineral based herbal formulation), Tailas (oil based herbal formulation) etc. Black pepper (*Piper nigrum L.*) is one of the commonly used ingredient in many Ayurvedic formulations and Piperine is the one of the major chemical constituent of black pepper (*Piper nigrum L.*)[3]

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It is generally possible to estimate a phytochemical marker for the ingredient plant raw material using various analytical techniques like TLC, HPLC and HPTLC. It is possible to detect the presence of the marker phytochemical compound and also quantify it to ascertain the limits in the final formulation[1,4,5].The matrix is dependent on the type of formulation and its excipients and has great influence on the correct quantitation of the phytoconstituent.

The current work is an attempt to develop a validated, simple, rapid and precise chromatographic method for quantitative determination of piperine from different types of Ayurvedic Polyherbal formulations like Pills (Arkavati), Mineral based herbal formulation (Kravyada Rasa), and Oil (Marichyadi taila)[6,7]. The method will be a quality control tool during routine quality analysis for these formulations.

2. MATERIALS AND METHODS

2.1 REAGENTS

For current work, HPLC grade methanol (Spectrochem) and double distilled water were used.

2.2 STANDARD

Stock solution of piperine (1000 ppm) was prepared in methanol using amber colored volumetric flasks. Piperine was obtained from Sigma Chemical Company, USA (99% purity). 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 4^0C protected from light and brought to room temperature before use. Piperine is light sensitive and isomerizes to isopiperine, chavicine and isochavicine[8].

2.3 AYURVEDIC FORMULATION

An Ayurvedic formulation, Arkavati (Pill) was prepared in our lab as per procedure mentioned in Ayurvedic formulary of India and this vati was used further for analysis[9]. Kravyada Rasa procured from Baidyanath Pharmaceuticals and Marichyadi taila from Nagarjun Pharmaceuticals (P) Ltd. Ahmedabad (Gujarat).

2.4 EXTRACTION PROCEDURE

0.5 gm powder of Arkavati and Kravyada Rasa were extracted using soxhlet apparatus in HPLC grade methanol (150ml) at 70^0C for 6hrs. The extract was filtered through whatman filter paper no. 1, filtrate collected and the final volume was made to 150ml with the help of HPLC grade methanol. Extraction of Marichyadi taila was done by adding 1ml of taila in 9ml of methanol. The mixture was vortexed for 30sec and kept overnight. Next day the upper methanolic layer was separated and used for further analysis.

2.5 INSTRUMENTATION AND CHROMATOGRAPHIC CONDITION

Chromatography was performed with Jasco's binary type high-performance liquid chromatography comprising two PU-1550 pumps with solvent mixing module HG-1580-31, 20µl loop and a Jasco multiwavelength detector MD-1510. A double-beam spectrophotometer was used for scanning and selecting the detection wavelength. Chromatograms were recorded by means of Borwin chromatography software version 1.21.

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 70:30 (v\v) delivered at a flow rate of 1.0 mL min^{-1} .The data was collected at wavelength of 342nm. Peak of

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piperine was identified by comparison with retention time of standard piperine (6.10 min).

2.6 METHOD VALIDATION

2.6.1 SYSTEM SUITABILITY

System suitability was determined by injecting, working standard solution of piperine (1000ng/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. (Table 1).

Table 1.

Summary of validation data

Method characteristic	Piperine
LOD	1 ng/ml
LOQ	10 ng/ml
Linear range (ng)	200ng/ml –3000ng/ml
Correlation coefficient (r)	0.9997
Slope	138.58
Intercept	1333.74
System suitability (RSD, %)	
Retention Time(n=5)	1.07
Area(n=5)	1.50
Precision (RSD, %)	
Inter-day (n = 3)	1.16–1.44
Intra-day (n = 3)	1.16–1.25

2.6.2 LIMIT OF QUANTITATION (LOQ) AND LIMIT OF DETECTION (LOD)

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. The LOQ of piperine was found to be 10 ng mL^{-1} and LOD was 1 ng mL^{-1}.

2.6.3 LINEARITY

Linearity was evaluated by analysis of working standard solutions of piperine of seven different concentrations. The peak area and

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concentration of piperine was subjected to regression analysis to calculate the calibration equations and correlation coefficients (Table 1). The response was linear in the range of 200 ng mL^{-1} to 3000 ng mL^{-1}

2.6.4 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 intra-day precision was repeated in the same manner on two more days by analyzing in triplicate (Table 1). The method was found to be precise.

2.6.5 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 (Table 2).The percent content of piperine was found to be 1.70%, 0.37% and 5.80% in the formulations Arkavati, Kravyada Rasa and Marichyadi taila respectively.

Table 2.

Assay Data

Formulation	Mean concentration	% content
Arkavati (n=7)	0.017±0.00013 gm/gm	1.70%
Kravyada Rasa (n=7)	0.0037±0.00001 gm/gm	0.37%
Marichyadi taila (n=7)	0.058±0.0009 mg/ml	5.80%

2.6.6 ACCURACY (RECOVERY)

To check the accuracy of the developed methods and to study the interference of formulation excipients, recovery experiments were carried out by standard addition method. A known amount of vati powder or taila sample was taken in seven different tubes for three different concentration level. To each tube known amount of piperine was added. Each sample was extracted and analysed by the developed HPLC method, in seven replicates and the amount of piperine recovered for each level, was calculated (Table 3).

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Table 3.

Summary of Recovery Data

Formulation	Mean % Recovery
Arkavati	99.17%
Kravyada Rasa	99.94%
Marichyadi taila	99.54%

3. RESULT AND DISCUSSION

Currently chemical markers or pharmacologically active components in Polyherbal formulation are employed for evaluating the quality and authenticity of Polyherbal formulation. Piperine is the active principle of black pepper (*Piper nigrum L.*), a ingredient of several polyherbal formulations.

There are several methods reported for quantitation of piperine[10,11,12,13 14]. But there are no reported methods for quantitation of piperine from Ayurvedic polyherbal formulations like Arkavati, Krvyadras and Marichyadi taila (Fig-3,4,5 and 6) reverse phase HPLC method. The current research paper is on quantitation of piperine from Ayurvedic formulations like Arkavati, Krvyadras and Marichyadi taila which has black pepper as one of the ingredients.

Piperine standard was detected and accurately quantified by using RP-HPLC with Methanol: Water, 70:30(v/v) as mobile phase (Fig-2). The identity of the piperine in the formulation was confirmed by overlaying the chromatogram obtained from the samples with that obtained from the piperine standard by comparing the retention time (5.8min).With the help of multiwavelength detector the spectra of standard Piperine peak was also compared with Piperine peak which comes from formulations. The regression equations obtained for piperine was y = 138.58x + 1333.7 (r=0.9997 n=7). The range of linearity was from 200 to 3000 ng $mL^{-1.}$ The results show that within the concentration range indicated there is an excellent correlation between peak area and concentration of piperine (fig.1). The correlation coefficient is 0.9997. The concentration of piperine in Arkavati was 17.00 mg/gm, in Krvyadras was 3.70 mg/gm and in Marichyadi taila was 0.058mg/ml. System suitability, intra-day assay precision and inter-day assay precision were measured. RSD values were less than 2%, indicating the method to be precise and reproducible (Table-1). The accuracy of the method was established by means of a recovery experiment which indicates method is accurate (Table-3).

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Fig. 1

Calibration curve for piperine.

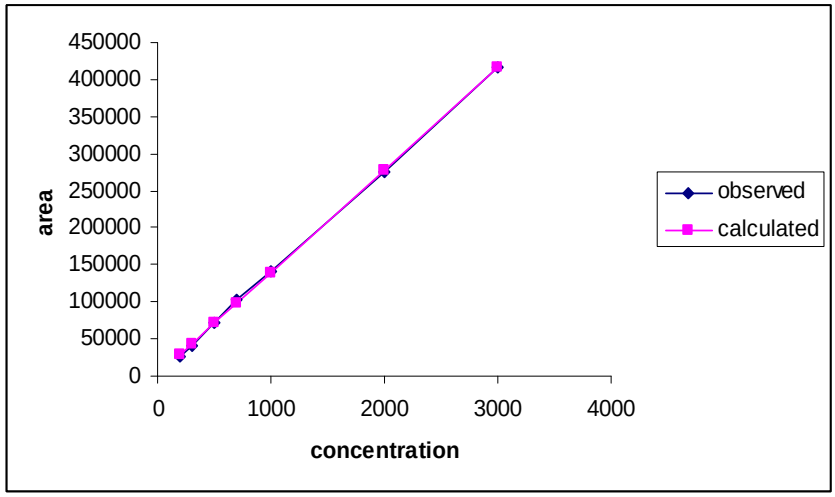

Fig 2

1000 ng/ml Piperine standard.

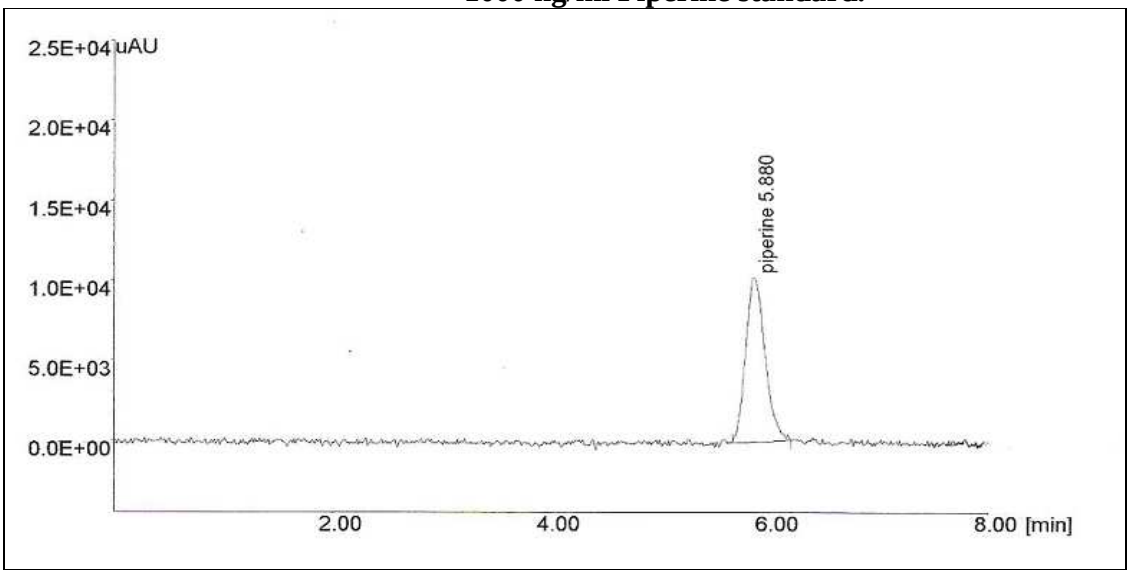

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Fig 3

Linearity for Piperine standard.

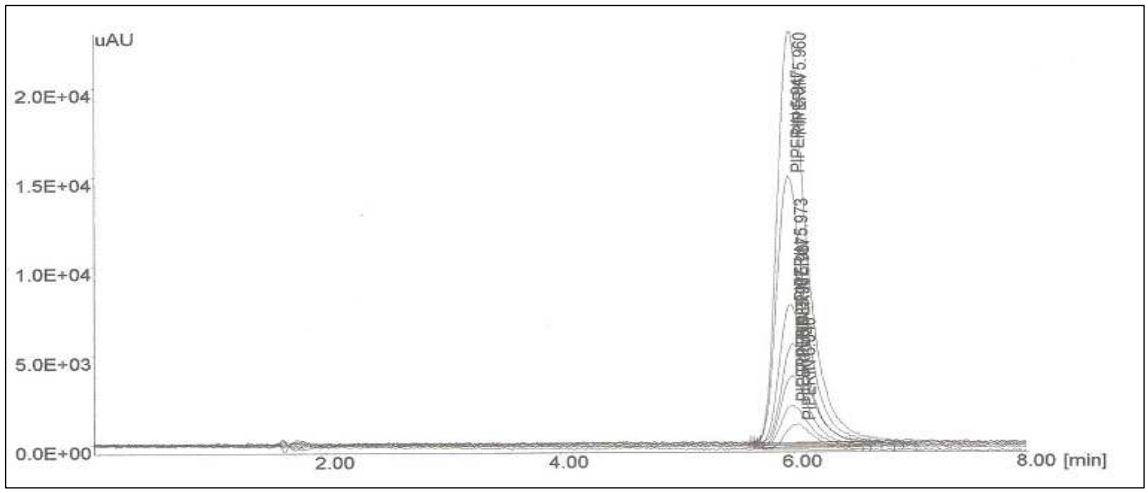

Fig 4

Piperine from Arkvati

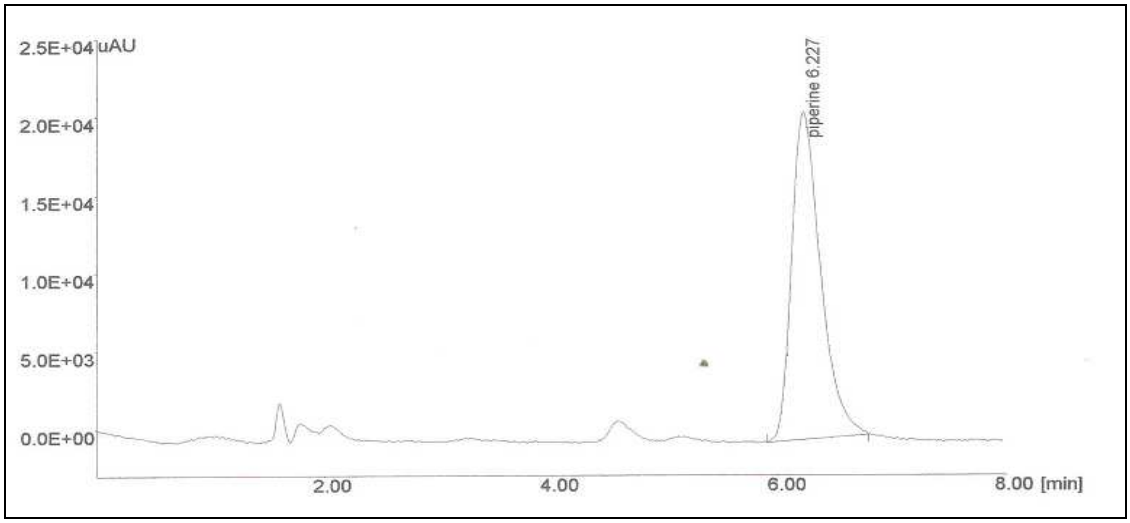

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Fig 5

Piperine from Kravyada Rasa

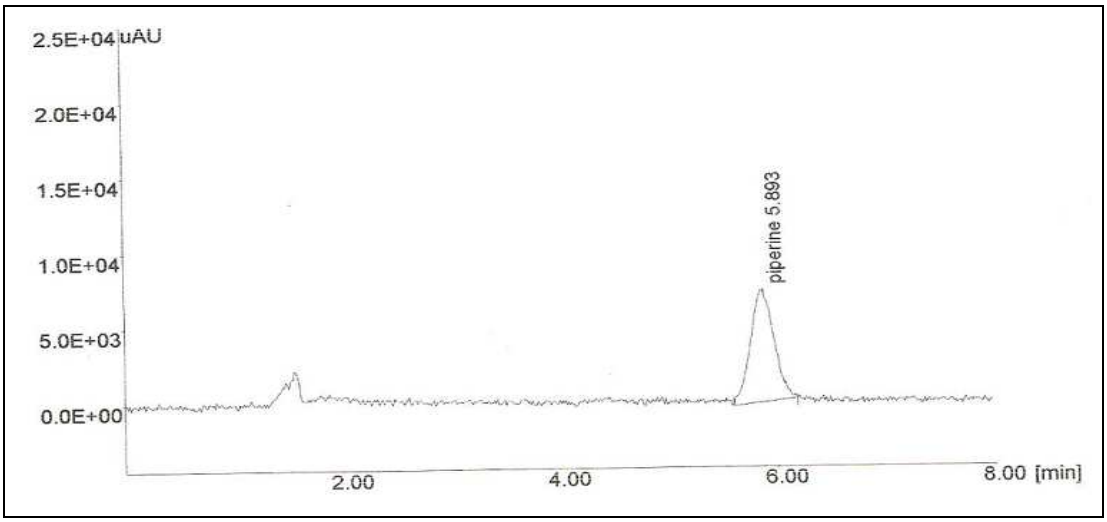
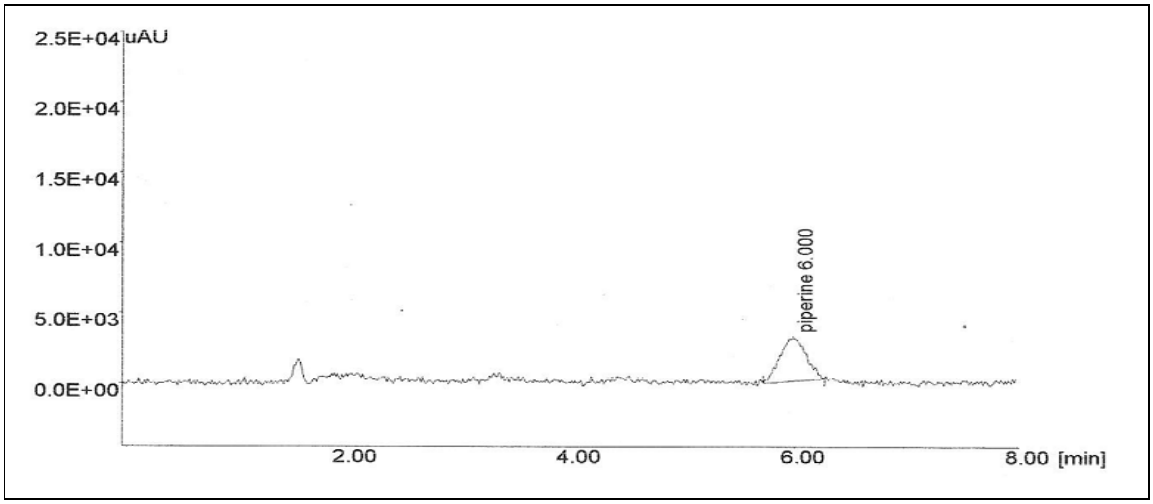


Fig 6

Piperine from Marichyadi taila.



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4. CONCLUSION

Literature survey[10,11,12,13,14] indicates that reported methods for detection and quantitation of piperine from *Piper nigrum* are time consuming because of the necessity of using buffers and are less economical because of the use of longer length of columns. There is no reported method for quantitation of Piperine from Ayurvedic formulations with different matrices of varied excipients. The Reverse phase HPLC method for quantitation of piperine reported in this work from different types of Ayurvedic polyherbal formulation i.e. Arkavati, Krvyadras and Marichyadi taila is sensitive, simple, fast and reliable for routine use in evaluating the quality of Polyherbal formulations with varied excipients. The developed method can be applied to various polyherbal formulations for the quantitation of piperine and used as a routine quality control method in pharmaceutical industries.

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