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Quantitative estimation of piperine in Pancasama churna by RP-HPLC

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ABSTRACT

Pancasama churna (PSC) is an Ayurvedic formulation containing Piper species (Piper longum) as main ingredients. This study was aimed to develop finger printing methods for well-known Ayurvedic formulation. Three batches of PSC were prepared in the laboratory and three different marketed formulations were procured from Ayurvedic medicine shop. A HPLC method was developed for the estimation of Piperine in laboratory and marketed formulations. The concentration of Piperine in raw material was found to be 1.41 ± 0.62 w/w in Piper longum fruits (pippli). The content of Piperine in laboratory formulations (PSC-I, II, III) were found to be 0.21 ± 0.006 , 0.23 ± 0.008 , and 0.22 ± 0.002 respectively and in different marketed formulations of PSC were PSC-A (0.25 ± 0.004 %), PSC-B (0.26 ± 0.006 %), PSC-C (0.27 ± 0.002 %) w/w respectively. In order to obtain precision and accuracy the recovery study was performed and result obtained with mean value 98.99%, which prove reproducibility of the result. This show significant precision of methods at 95% confidence level. The mean of % RSD value was found to be 0.094 with the mean standard error 0.036. Results of statistical analysis show present HPLC method for determination of Piperine is simple, precise, accurate and suitable for routine analysis of Piperine in PSC. The developed fingerprints can be used as a standard and Piperine can be used as a possible marker compound for fingerprinting of PSC.

Keywords: Fingerprints, Pancasama churna (PSC), Piper species, Piperine, marker, HPLC.

INRODUCTION

Ayurveda is a science dealing not only with treatment of some diseases but is a complete way of life. This indian system of medicine has laid down principles and methods of treatment for various diseases including chronic illness where there is no definite treatment, and sympatomatic

relief is the only exiting treatment option^{1,11}. Chromatographic fingerprint have been suggested to check for authenticity or provide quality control of herbal medicine . Chromatography has the advantage of separating a complicated System into relatively simple sub-systems and then presenting the chemical patterns of herbal medicine in the form of a chromatogram^{2,12} World Health Organization (WHO) has emphasized the need to ensure the quality of medicinal plant products by using modern controlled technique and applying suitable standards . For standardization of natural product drugs, single chemical entities, “marker compounds,” may be used as potency standards in high performance liquid chromatography (HPLC) analysis¹⁰. Using well-characterized marker compounds, conventional pharmaceutical manufacturing criteria for assay and content uniformity may be applied. Fingerprints can be a unique identification utility for herbs and their different species, and can be used for modeling pharmaceutical activities. Now, chromatographic fingerprint technique plays an important role in controlling the quality of samples and focusing on the identification and assessment of the stability of the components . HPLC analysis for marker compounds may provide additional information in the form of chromatographic fingerprints. The present study is undertaken to develop certain fingerprints for an Ayurvedic formulation. Pancasama churna used in digestive impairment, flatulence, abdominal pain, rheumatic arthritis, piles and other abdominal disorders. Ayurvedic formulary of India has given the specification for the composition of PSC, it should contain piper species as a major ingredient apart from different herbs and salts^{3,4}.

Aims and Objectives

Pharmacopoeial standards for Ayurvedic formulations published by the Central Council for Research in Ayurveda and Siddha gives certain physical parameters as standards for churna, these standards are not based on modern analytical methods⁵. It is therefore essential to develop definite and accurate analytical tools to as certain consistency and quality of Ayurvedic preparation from batch to batch in pharmaceuticals which may results in acceptability world wide. In present study we tried to develop a method that serves as fingerprinting method for Pancasama churna(PSC).

MATERIALS AND METHODS

Experimental

All the solvents for HPLC analysis were HPLC grade and purchased from E. Merck and S. D. Fine Chemicals, Mumbai. All solvents used for extraction were primarily distilled before use. SHIMADZU – LC 10 AT HPLC was used for piperine analysis. All the results are obtained by repetition of the each experiment six times (n= 6).

Procurement of drug

Crude drugs were procured from local market and identified by macroscopic and microscopic characters^{6,7,8}.

Preparation of formulations

1. Three batches were prepared in laboratory (named as PSC-I,PSC-II and PSC-III) according to strict methods of ‘Ayurvedic formulary of India’ and Sarangadhara-samhita.
2. Commercially available brands PSC-A, PSC-B, and PSC-C, of Pancasama churna were procured from local market.

Sample preparation for estimation of Piperine content

1.5 gm Pancasama churna was refluxed for 1 hour with 100 ml of methanol. The volume were reduced under pressure and filtered by 0.2 μm membrane filters. The filtrate was diluted up to 100 ml with methanol. To the 20 ml of resulting solution, 2 ml of 0.5-mg/ml solution of p-dimethyl amino benzaldehyde (internal standard) was added, and made the final volume 25 ml with methanol ^{7,12} [Fig. 1].

Preparation of standard solution

Piperine was purchased from Lancaster, England. Standard solution was prepared by the addition of 2 ml of solution a (1mg/ml) of Piperine and 2 ml of internal standard solution (0.5 mg/ml of p-dimethylamino benzaldehyde) in a 25 ml volumetric flask made the final volume to 25 ml with methanol.

HPLC studies

Estimation of Piperine was carried from different batches (three marketed and laboratory batch) of PSC with following conditions:

Column : C 18 (25cm X 4.6 mm i.d.) 10 μ ,
mobile phase : methanol: water (69:31),
detection : at 343 nm (reference wavelength: 343 nm),
injection volume : 20 μl and
flow rate : 1.5ml/min.

Calibration

The Piperine content of PSC was determined using a calibration curve established with seven dilutions, at concentrations ranging from 0.5-20 $\mu\text{g/ml}$. Each concentration was measured in triplicate. The corresponding peak areas were plotted against the concentration of the Piperine injected. Peak identification was achieved by comparison of both the retention time and UV absorption spectrum with those obtained for standards.

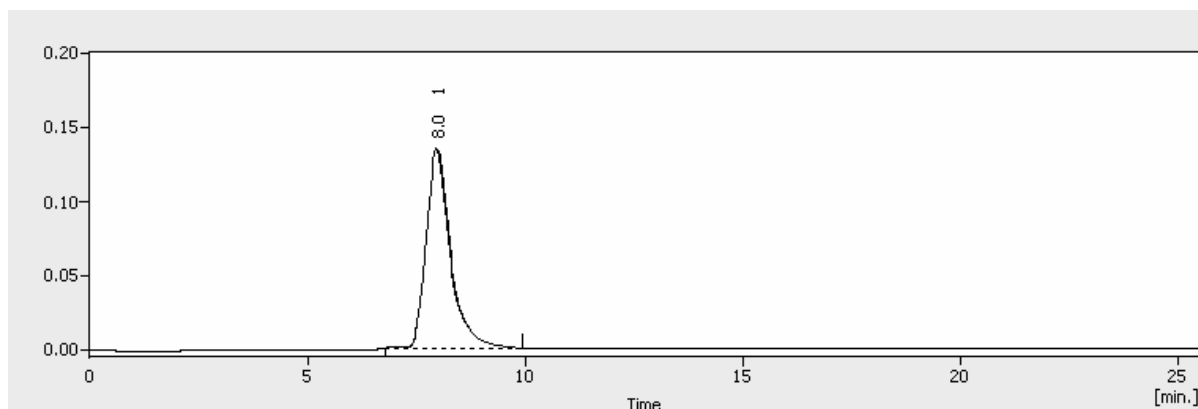


Figure:2. RP-HPLC Chromatogram of standard Piperine at 343 nm

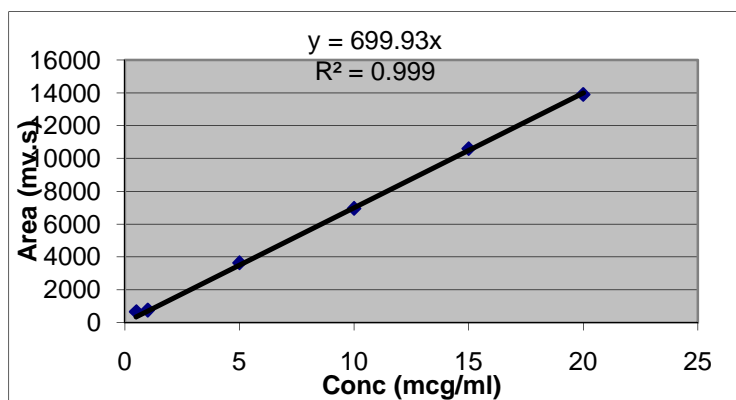


Figure 1: Calibration curve of standard Piperine

Validation parameters

Selectivity and peak purity

Selectivity was checked by using prepared solutions of PSC and available standards optimizing separation and detection. The purity of the peaks was checked by multivariate analysis. The three spectra corresponding to up slope, apex and down slope of each peak were computer normalized and super imposed. Peaks were considered pure when there was a co-incidence between the three spectra (match factor was =98%).(Table-I)

Linearity, limits of detection and quantification

The linearity of the detector response for the prepared standards was assessed by means of linear regression regarding the amounts of each standard, measured in μg , and the area of the corresponding peak on the chromatogram. Linearity was also confirmed for PSC prepared sample solutions. After chromatographic separation, the peak areas obtained were plotted against concentrations by linear regression. Limits of detection and quantification were determined by calculation of the signal-to- noise ratio. Signal-to-noise ratios 3:1 and 10:1 were used for estimating the detection limit and quantification limit, respectively, of the method.

Precision

The repeatability of the injection integration was determined for both standard piperine and the content of piperine in Pancasama churna. A standard solution containing reference compounds and prepared sample solutions was injected. Pancasama churna samples were also prepared 2 times to evaluate the repeatability of the process. The mean amount and R.S.D. values were calculated. The precision was calculated at two different concentrations high and low tested in the concentration range. For standardization the sample was injected at eight different concentrations and linearity was noted [Table-1].

Accuracy

The accuracy of the method was determined by analyzing the percentage of recovery of the piperine in the Pancasama churna. The samples were spiked with two different amounts (100, 150 μg) of standard compounds before sample preparation. The spiked samples were extracted by triplicate and analyzed under the previously established optimal conditions. The obtained average contents of the target compounds were used as the “real values” to calculate the spike recoveries [Table-1].

Robustness

For the determination of the method's robustness a number of chromatographic parameters, such as column package and size, mobile phase composition and gradient ratio, flow rate and detection wavelength, were varied to determine their influence on the quantitative analysis. Interday and intraday variability was studied for the sample, by injecting the same concentration of the sample on three different days and the standard error mean was calculated.

Statistics

When applicable, one-way or two-way analyses of variance (SPSS11.0 for window) were used to assess the observed differences in the Piperine content. Differences were considered to be statistically significant when the P-value was <0.05 .

RESULTS AND DISCUSSION

In the present study, spectral and chromatographic studies were performed. Results of the The RP-HPLC analyses of PSC were performed, samples were injected at seven different concentrations and the linearity was observed with in the concentration range of 0.5-20 $\mu\text{g/ml}$ [Fig. 1]. Piperine was well separated at retention time 8.020 respectively. The concentration of Piperine present in raw material was found to be 1.41 ± 0.62 w/w in Piper longum fruits (pippli). The content of Piperine in laboratory formulations (PSC-I, II, III) were found to be 0.21 ± 0.006 , 0.23 ± 0.008 , and 0.22 ± 0.002 respectively and in different marketed formulations of PSC were, for PSC-A (0.25 ± 0.004 %), PSC-B ($0.26\pm 0.006\%$), PSC-C (0.27 ± 0.002 %) w/w respectively [Table-2, Fig-2]. The HPLC method was validated by defining the linearity, peak purity, limit of quantification and detection, precision, accuracy, specificity and robustness. For the qualitative purposes, the method was evaluated by taking into account the precision in the retention time, peak purity, and selectivity of piperine elutes. A high repeatability in the retention time was obtained with (R.S.D.) value lower than for both standard and samples even at higher concentration. (Table-3).The peak purity was studied in the major peaks. Linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision were evaluated for quantitative purposes [Table-1].Thus LOD and LOQ found to be 0.063, 0.071mg/ml respectively which suggest full capacity for quantification of piperine content in different laboratory and marketed batches of PSC. R^2 value for the regression equation of the Piperine was higher than 0.9988 thus confirm the linearity of the method. The recovery study was performed at two levels by adding known amount (100, 150 μg) of piperine with reanalyzed sample of PSC found to be close to 98.99(mean)% and a higher repeatability indicate a satisfactory accuracy in the proposed methods [Table-3].Finally the robustness of the method was also assessed. Minor modification of the initial mobile phase gradient (from 25 to 30% solvent instead of 31%) had no effect on the peak resolution of the compound. Therefore, this HPLC method for fingerprinting of PSC can be regarded as selective, accurate, precise, and robust. The method is very adaptable because of the precision and repeatability for the traditional Ayurvedic formulation like PSC and suitable for routine analysis of Piperine in PSC. There was not much variation in the interday and intraday injections performed with the mean of % RSD value was found to be 0.094% with the mean standard error 0.036 respectively. Piperine estimation can be utilized as a possible analytical marker for fingerprinting of Pancasama churna.

Table-I Validation parameter (Mean% \pm SD,n=3)

Sno.	parameter	value
1	Absorption maxima	343
2	Bee's law limit	0.5-20ug/ml
3	Regression equation(y=bx+a)	699.9x
4	Intercept(a)	0
5	Slope(b)	699.9
6	Correlation coefficients(r ²)	0.999
7	LOD mg/ml	0.063
8	LOQ mg/ml	0.071
9	Precision (n=6, % RSD)	0.094
10	Accuracy(%)	98.99

Table-2 Estimation of piperine (Mean% \pm SD,n=3)

s.no.	name	Piperine content% w/w
01	piper longum(pippli)	1.41 \pm 0.62
02		PSC-I 0.21 \pm 0.006
03		PSC-II 0.23 \pm 0.008
04	PANCASAMA CHURNA	PSC-III 0.22 \pm 0.002
05		PSC-A 0.25 \pm 0.004
06		PSC-B 0.26 \pm 0.006
07		PSC-C 0.27 \pm 0.002

Table-3 Recovery study (Mean% \pm SD,n=3)

S.no.	Amount of piperine(microgm/ml)			RSD%	SE	Recovery%
	In sample	added	estimated			
01	100	100	197.2 \pm 0.12	0.0608	0.021	98.50
02	200	150	348.2 \pm 0.42	0.120	0.050	99.49
mean				0.094	0.036	98.99

Mean \pm SD of six determinations,
SE=Standard error

RSD= Relative standard deviation

CONCLUSION

The developed high performance liquid chromatographic method for estimation of Piperine from Pancasama churna could be used as a valuable analytical tool in the routine analysis, to check the batch to batch variation. Estimation of Piperine can be used as one of the appropriate analytical markers for the finger printing.

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