Standardization Study of Antifertility Drug - Pippalyadiyoga

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Abstract: The present paper deals with the standardization study of pippalyadiyoga powder. It is used as a long acting contraceptive. The standardization of compound drug has been achieved by physico-chemical analysis and high performance liquid chromatography (HPLC) fingerprint studies. Quantitative evaluation of borax in pippalyadiyoga showed 19.08% as sodium borate. 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 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. Quantitative evaluation of piperine in pippalyadiyoga was found to be 0.339%.

Key words: Standardization, Pippalyadiyoga, HPLC and Antifertility Drug.

Introduction

Pippalyadiyoga is a herbo-mineral ayurvedic drug used as an antifertility formulation. The herbal components are pippali (*Piper longum* Linn., Piperaceae) and vidanga fruits (*Embelia ribes* Burm. f., Myrsinaceae) and a mineral component tankana (Borax) present in 1:1:1 ratio by weight. It is a long acting oral contraceptive¹. The contraceptive effect of pippalyadiyoga was assessed which showed a good response and high antifertility effect². Surva Mandal *et al.*, have reported the organoleptic study, isolation and quantitative estimation of the active principles and thin layer chromatography study of pippalyadiyoga and its herbal components³. The piperine extracted from *Piper nigrum* Linn. was selected as the standard for identification of pepper in finished products or ayurvedic medicines. Piperine was subjected to TLC study along with finished products⁴. HPTLC study was performed for quantitative determination of piperine content from *Piper nigrum* Linn.⁵. So, in the present paper the physico- chemical and HPLC fingerprint study has been discussed.

Materials and Methods

Pippalyadiyoga powder was supplied by the Head quarter of CCRAS (Central Council for Research in Ayurveda and Siddha), New Delhi, for chemical analysis and standardization study. Standard piperine was procured from M/s Sigma –Aldrich Chemie GmbH, Germany.

Physico-chemical analysis

The organoleptic study, pH (5% aqueous), moisture content, total ash, alcohol soluble extractive, water soluble extractive, qualitative organic, inorganic and heavy metal (lead and arsenic) analysis and quantitative analysis of borax were carried out as per the standard procedures⁶⁻⁸.

Preparation of sample

The 5% alcoholic extract of compound drug was prepared by soaking the material for 18 hr in absolute alcohol. The extract was centrifuged at 3000 rpm and then filtered through 0.2 μ membrane filter using high-pressure vacuum pump.

Calibration

The standard piperine solution was prepared by dissolving 5 mg of piperine in 5 ml of absolute alcohol. Three additional calibration levels were prepared by diluting this solution with alcohol to obtain 0.005%, 0.01%, 0.02% and 0.1% concentrations for studying the linearity. The precision of the method was also studied by injecting a sample solution of concentration 0.01% five times and finding out the standard deviation and coefficient of variation.

HPLC analysis

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 5 μ 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 0.2 μ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 250 – 260 kgf / cm². The initial B-pump concentration was 50%. The column temperature was maintained at 27°C. 20 μ l of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton).

Results and Discussion

The analytical data of pippalyadiyoga is tabulated in the **Table-1**. The powder of pippalyadiyoga was brown in color with aromatic odor and astringent taste. The pH of 5% aqueous solution was found to be 9.0, moisture content 3.87%, total ash content 30.15%, alcohol soluble extractive 9.98% and water soluble extractive 42%. The qualitative organic analysis of alcoholic extract of pippalyadiyoga powder showed the presence of alkaloids, glycosides, steroids, tannins and phenolic compounds. The powder showed the presence of calcium, iron, phosphate, sodium, magnesium, chloride, potassium, carbonate and borate. The assay for borax in pippalyadiyoga showed 19.08% as sodium borate. Heavy metal analysis showed negative result for arsenic and lead. It has been reported that the organoleptic characters of pippalyadiyoga showed grey color with pungent, bitter and astringent taste. The pH of aqueous solution of pippalyadiyoga reported to be 9 –10 (Universal pH paper)³.

Standard piperine solutions of 0.005%, 0.01%, 0.02% and 0.1% concentration analyzed for studying the linearity and the area count obtained for these solutions are presented in **Table-2**. 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-3**) 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.

The HPLC chromatogram of standard piperine at an optimum wavelength of 345nm showed a mean area (**Table-2**) of 7328514.2 at a mean retention time of 23.733min (**Figure-1**). The recovery

value of standard piperine was 99.4%. The HPLC chromatogram of pippalyadiyoga corresponding to standard piperine showed at a retention time of 23.061 min with an area of 9944671 at a wavelength of 345 nm (**Figure-2**). Quantitative evaluation of piperine in pippalyadiyoga showed 0.339%. The HPLC analysis of methanolic extract of piperine isolated from *Piper nigrum* Linn. showed optimum wavelength at 343nm³.

Table 1. Data of physico-chemical analysis of pippalyadiyoga

S. No.	Parameters	Pippalyadiyoga
1.	Color	Brown
2.	Odor	Aromatic
3.	Taste	Astringent
4.	pH (5% aqueous)	9.0
5.	Moisture (%)	3.87
6.	Ash (%)	30.15
7.	Alcohol Soluble Extractive (%)	9.98
8.	Water Soluble Extractive (%)	42
9.	Borax content (%)	19.08
10.	Alkaloid	+ve
11.	Glycoside	+ve
12.	Steroid	+ve
13.	Tannin	+ve
14.	Phenol	+ve
15.	Calcium	+ve
16.	Iron	+ve
17.	Phosphate	+ve
18.	Sodium	+ve
19.	Magnesium	+ve
20.	Chloride	+ve
21.	Potassium	+ve
22.	Carbonate	+ve
23.	Borate	+ve
24.	Arsenic	nil
25.	Lead	nil

Table 2. 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

Figure 1. HPLC chromatogram of standard piperine **Figure 2.** HPLC chromatogram of pippalyadiyoga **Table 3.** 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

Conclusion

The physico-chemical data and HPLC fingerprint profiles were evolved for pippalyadiyoga can be considered for fixing up standards.

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