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Quantitative Estimation of Piperine in Trikatuku Curanam

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Curanams are important group of formulations used by ayurvedic and siddha physicians to treat various types of diseases. Trikatuku curanam which is used for the treatment of various types of gastric disorders is prepared as per the formula given in Siddha Formulary of India, Part I, First Edition, by procuring all the drugs from the local market. In the present study, an attempt has been made to develop a HPTLC method of quantitative estimation of piperine in laboratory prepared authentic formulation and a marketed formulation of Trikatuku curanam. The two formulations were subjected to methanol and ethyl acetate extractions by soxhylation. Piperine was quantified in the above two extracts by using high performance thin layer chromatography. The detection and quantification was performed at a wavelength of 340 nm. The laboratory formulation was found to contain 1.491 % of piperine while the commercial formulation shows 1.904 % of Piperine in methanol extracts. The laboratory formulation was found to contain 1.820 % of piperine and commercial formulation shows 2.432 % of piperine in ethyl acetate extracts. The method was validated by carrying out linearity, percentage recovery and reproducibility results. Linearity studies indicated that piperine was in the linear range of 30-105 ng while the % recovery studies revealed a recovery of 98.73 % w/w, thus proving the accuracy and precision of the analysis.

Key Words: HPTLC, Trikatuku curanam, Piperine, Laboratory formulation, Commercial formulation.

INTRODUCTION

Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the ayurvedic or siddha formulations is the lack of standard quality control profiles[1]. The quality of herbal medicine, that is, the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and therefore, modern analytical techniques are expected to help in solving this problem.

Trikatuku curanam is a herbal formulation used extensively in siddha system of Indian medicine to treat various gastric disorders. Standardization of ayurvedic or siddha formulations is the need of the day. Many of them do not have standard identification tests or analytical procedures to maintain their quality. Hence, modern methods can be used to set up certain standards for the herbal formulations. Trikatuku curanam formulation consists of black pepper (*Piper nigrum*), long pepper (*Piper longum*) and dried ginger (*Zingiber officinalis*). The pharmacopoeial standards in ayurvedic or siddha pharmacopoeia are not adequate enough to ensure the quality of plant drugs or their formulations. Therefore, the formulations were subjected to HPTLC analysis.

EXPERIMENTAL

A Camag HPTLC system equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner III, Reprostar and Wincats 4.02, an integration software (Switzerland).

Analytical grade toluene, ethyl acetate and methanol were obtained from S.D. Fine Chem. Ltd. (Mumbai, India). Pure piperine was obtained from Sigma Aldrich Ltd., (Bangalore, India). Pre-coated silica gel 60 F_{254} TLC aluminium plates (10 × 10 cm, 0.2 mm thick) were obtained from E. Merck Ltd. (Mumbai, India).

Drugs: Black pepper (*Piper nigrum*), long pepper (*Piper longum*) and dried ginger (*Zingiber officinalis*) were collected from the local market and authenticated by the Department of Pharmacognosy, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The commercial formulation, Trikatuku curanam was obtained from Indian Medical Practitioners' Co-operative Pharmacy (IMPCOPS), (Chennai, India). HPTLC method for estimation of piperine[2].

Preparation of standard piperine solution: A stock solution of piperine (1 mg/mL) was prepared by dissolving 10 mg of accurately weighed piperine in methanol and making up the volume to 10 mL with more methanol in amber coloured volumetric flask covered with aluminium foil, since piperine in solution isomerizes to isopiperine, chavicine and isochavicine on exposure to light[3]. The stock solution was further diluted with methanol to give a standard solution of piperine (15 ng/mcl). Another standard solution of piperine was prepared with ethyl acetate solvent in the same manner. Two standard solutions with different solvents were prepared to ascertain the type of solvent best suitable to produce a single sharp peak for piperine[4].

Chromatographic conditions: Stationary phase: Pre-coated silica gel 60F_{254} TLC plate (10 × 10 cm, 0.2 mm thickness); mobile phase: toluene: ethyl acetate (7:3 v/v); saturation time: 15 min; wavelength: 340 nm; lamp: deuterium.

Calibration curve for standard piperine: The standard solutions (30 to 105 ng per respective spot) were applied in triplicate on TLC plate. The plate was developed and scanned as per the chromatographic conditions mentioned above. The peak areas were recorded. Calibration curve of piperine was prepared by plotting peak areas vs. concentrations of piperine applied (Fig. 1).

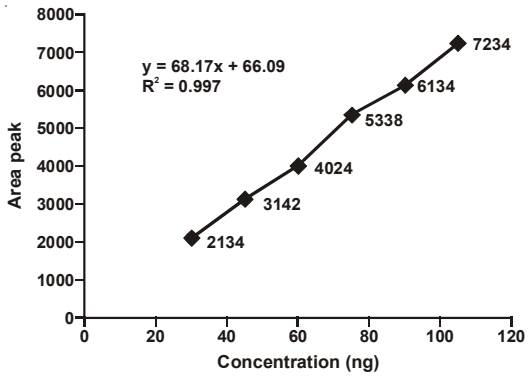

Fig. 1. Calibration curve of piperine

Preparation of Trikatuku curanam: Trikatuku curanam was prepared in the laboratory as per the formulation given in the Siddha Formulary of India[5]. Formulation:

Siddha formulary name	Synonym	Botanical name	Quantity
Milagu	Black pepper	*Piper nigrum*	1 Part
Thippili	Long pepper	*Piper longum*	1 Part
Chukku	Dried ginger	*Zingiber officinalis*	1 Part

The individual drugs were powdered separately and sieved through a fine mesh. Then the required quantities by weight were taken and thoroughly mixed to uniformity.

Preparation of extracts: The laboratory formulation samples (10 g each) and the commercial formulation samples (10 g each) of trikatuku curanam were extracted for 6 h by using two different solvents, methanol and ethyl acetate in a soxhlet apparatus. All the four extracts were then concentrated at a low temperature, filtered through Whatmann filter paper No.1 and the final volumes were made up to 10 mL with more respective solvents. The solutions were further diluted to produce a uniform concentration of 10 mcg/mcl of the samples.

Method specifications: 5 mL each of methanolic and ethyl acetate extracts of LF and CF of Trikatuku curanam and 5 mcl of standard piperine

solution were applied on the precoated TLC plates. The samples were applied as 6 mm bands using automatic Camag Linomat V applicator with sufficient N_2 flow. The mobile phase used was as mentioned above. The plates were developed in a twin trough chamber to a distance of 8 cm under chamber saturation conditions. After development the plates were dried in air and scanned at 340 nm.The plates were photographed at 254 and 366 nm by using Camag reprostar instrument. The contents of piperine in the laboratory formulation and commercial formulation of methnolic and ethyl acetate extracts were determined by comparing the area of the chromatogram of the above formulations with the calibration curve of piperine.

RESULTS AND DISCUSSION

Standard piperine in ethyl acetate showed a single peak in HPTLC chromatogram. Calibration curve of piperine was prepared by plotting different concentrations of piperine *vs.* average area of the peak. The formulation samples were analyzed by the proposed method. The amount of piperine was computed from the above calibration curves.

The data from Table-1 revealed that the laboratory formulation was found to contain 1.491 % of Piperine while the commercial formulation shows 1.904 % of piperine in methanol extracts. The laboratory formulation was found to contain 1.820 % of piperine and commercial formulation shows 2.432 % of piperine in ethyl acetate extracts. Thus it was observed that though, both the formulations contained satisfactory amount of piperine, the marketed formulation of Trikatuku curanam showed a higher content of piperine.This may be due to the procurement of crude drugs from different geographical location by the manufacturer. It is also revealed from the data that ethyl acetate is a better solvent to extract piperine from the formulations than that of methanol as proved by the content of piperine in ethyl acetate extracts. Further, the ethyl acetate extracts of both the formulations have shown maximum number of peaks in the chromatogram. Similarly, the number of peaks in the chromatogram of laboratory formulation and commercial formulation are same (5 Nos) indicating that the active principles present in the two formulations are more or less same. The standard piperine in ethyl acetate showed only one sharp peak when compared to piperine in methnol which showed more than one peak. This may be due to quick isomerization of piperine in methanol.

Validation of HPTLC method

Linearity: A representative calibration curve of piperine was obtained by plotting the peak area of piperine (30-105 ng). The correlation coefficient for piperine was found to be 0.997 and thus exhibits good linearity between concentration and area (Table-2).

TABLE-1
PERCENTAGE OF PIPERINE IN DIFFERENT FORMULATION OF TRIKATUKU CURANAM

TKC formulations	Piperine (% w/w)
Laboratory formulation – Methanol extract	1.491
Laboratory formulation – Ethyl acetate extract	1.904
Commercial formulation – Methanol extract	1.820
Commercial formulation – Ethyl acetate extract	2.432

TABLE-2
RESULTS OF METHOD VALIDATION

Analytical Method	Accuracy (% recovery)	Precision (SD)	Linearity (ng)	Coefficient of variation % (CV)
HPTLC of piperine	98.73	0.1465	30-105	0.498

Accuracy (recovery %): The percentage recovery of piperine was found to be 98.73 which is highly satisfactory (Table-3).

TABLE-3
RESULTS OF RECOVERY STUDY OF THE METHOD FOR PIPERINE

Amount of sample taken (mg)	Amount of piperine in A (mg)	Amount of piperine added to A (mg)	Amount of piperine taken B+C (mg)	Total piperine found (mg)	% Recovery E/D × 100
A	B	C	D	E	
1100	22.26	2	24.26	24.04	99.03
1200	22.38	5	27.38	27.10	98.37
1300	22.58	10	32.58	32.16	98.71

Average recovery: 98.73 %

Specificity: It was observed that other constituents present in the formulations did not interfere either with the peak of piperine. Therefore the method was specific. The spectrum of standard piperine spots and piperine present in the samples were found to be similar or overlap.

Limit of detection: The minimum detectable limit was found to be 15 ng/spot for piperine.

Conclusion

The proposed HPTLC method has been validated as per ICH guidelines[6] and was found to be rapid, simple and accurate for quantitative estimation of piperine from different formulation extracts. The percentage

recovery value of piperine which is 98.73 shows the reliability and suitability of the method. The method was found to be useful in detecting the genuiness of the formulation and thus suitable to evaluate various formulations available in the market.

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