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RESEARCH ARTICLE

HPTLC QUANTIFICATION METHOD OF PIPERINE AND ZINGIBERENE IN A VETERNARY POLYHERBAL FORMULATION

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ABSTRACT

Objective: Standardization of herbal formulations in terms of quality and composition of raw materials are important to ensure quality and optimum levels of active principles. HPTLC has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds. An Ayurvedic veterinary formulation used as effective appetite enhancer, in the animals suffering from anorexia, off-feed & ulcers. It contains *Zanthoxylum alatum* DC, *Piper longum* Linn, *Piper nigrum* Linn, *Ferula narthex* Bioss, *Cuminum cyminum* Linn, *Carum copticum* Linn, *Zingiber officinale* Roscoe. The need of the ours is to evolve a systematic approach and to develop well-designed methodologies for the standardization of herbal raw materials and herbal formulations.
Method: The present study was to develop the fingerprinting and quantification methods for the formulation by simple high-performance thin layer chromatography (HPTLC) determination using piperine and zingiberene as standard, which is an important and major content in formulation. HPTLC quantification methods for determination of piperine and gingiberine from the market formulation and lab formulation had been developed.
Result: The concentration of piperine present in raw lab and market formulation was found to be 3.7% w/w and 3.15% w/w respectively and that of for zingiberene was found to be 9.04% and 10.36% respectively.
Conclusion: Lab and market both the formulation were up to the standard.

INTRODUCTION

Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker based standardization. HPTLC has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability for high throughput screening etc.[1] The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. An ayurvedic veterinary formulation used as effective appetite enhancer, in off-feed, anorexia, poor digestion and general debility. It contains *Zanthoxylum alatum* DC, *Piper longum* Linn,, *Piper nigrum* Linn., *Ferula narthex* Bioss, *Cuminum cyminum* Linn., *Carum copticum* DC, *Zingiber officinale* Rosc.

MATERIAL AND METHOD[2,3,4,5,6]

Marketed formulation is formulated by Mac ayurcare, Khambhat, Gujarat. All the fresh raw material and finished product was provided by Mac ayurcare, Khambhat, Gujarat All the raw materials were authentified by Dr. M. S. Jagid, department of botany, Sir P. T. Science College, Modasa, Gujarat.

Selection of mobile phase

Selection of mobile system for the method development of the formulations was done by trial and error methods. Stationary Phase: Precoated silica gel G_{60} plate.

Sample preparation

Lab. formulation and market formulation

- 50 g. of avleha was extracted exhaustively with 50 ml methanol for 1 hour and then filtered.
- The filtrate was concentrated.
- 1 gm of residue was mixed with 100 ml ethanol and from that mixture 1 ml of mixture taken and diluted up to 100 ml (100µg/ml)

- Both laboratory formulation and market formulation were prepared using same dilutions, and used for further studies.

Preparation of standard solution of piperine (100µg/ml)

1 mg of pure piperine (Sigma Aldrich Ltd) was taken and dissolved in 10ml ethanol (stock solution). 1 ml of stock solution diluted up to 10 ml which was used for further studies.

Preparation of standard solution of Zingiberene (100µg/ml)

1 mg of pure Zingiberine (gift sample from Paras Pharma) was taken and dissolved in 10ml ethanol (stock solution). 1 ml of stock solution diluted up to 10 ml which was used for further studies.

Calibration

0.5, 1, 1.5, 2, 2.5 and 3 µl of piperine and 14, 16, 18, 20, 22, 24 µl of Zingiberene were spotted on 10×10cm precoated Silica gel G_{60} F_{254} plate was developed in Toluene : Ethyl acetate (7:3) mobile phase using twin trough chamber.

Detection

All the chemical markers were detected under U.V. light. i.e Piperine at 340 nm and Zingeberene at 420 nm

RESULT

Separation and Quantification of Piperine and Zingiberene

Table 1. Trial and error method for the selection of Solvent system

Toluene	Ethyl acetate	Result
7	0.3	Tailing
7	1	Tailing
7	2	Tailing
7	2.5	Spreding
7	3	All components separates

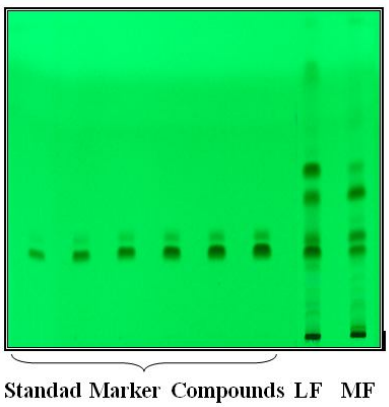


Figure 1. HPTLC plate for the Standard piperine with LM and MF

Calibration curve for piperine

Calibration curve of Piperine and Zingiberene was developed in the mobile phase system Toluene : Ethyl acetate (7:3) which was selected by trial and error method. (Table 1) HPTLC plate for the Standard piperine with LM and MF shown in Figure 1 and Densitometric Chromatogram of Piperine Standard shown in Figure 2. The results showed linearity and correlation coefficient within the range of concentration (1µg/ml-6µg/ml). There was good correlation between peak area and the corresponding concentration of Piperine as shown in Figure 3. The best fitting liner equation was y = 53.53x + 22297 (R2=0.993). HPTLC plate for the Standard Zingiberene with LM and MF shown in Figure 5 and Densitometric Chromatogram of Zingiberene Standard shown in Figure 6. The results showed linearity and correlation coefficient within the range of concentration (1µg/ml-6µg/ml). There was good correlation between peak area and the corresponding concentration of Piperine as shown in Figure 7. The best fitting liner equation was y = 47.82x + 81119 (R2=0.992).

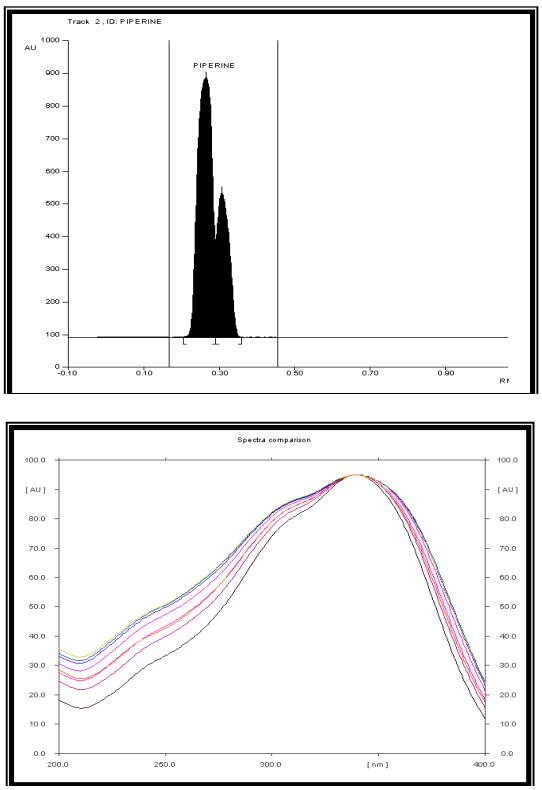


Figure 2. Densitometric Chromatogram of Piperine Standard

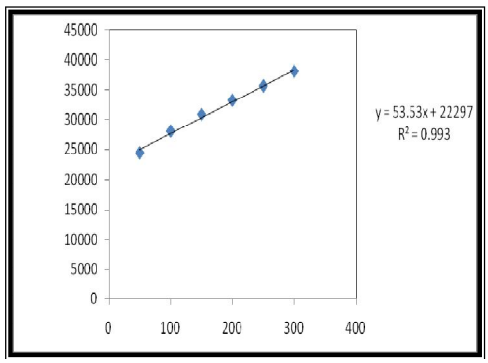


Figure 3. Calibration curve of Piperine

Estimation of Marker compounds in LF and MF

The peak area of marker compounds in formulation was seen in calibration spectrum data. The percentage of marker compounds in different sample was calculated and shown in Table 2.

DISCUSSION

From the results it can be observed that the amount of piperine is more in LF as compared to MF.This indicates that the raw materials used in the MF are not containing the specified

Table 2. Estimation of Marker compounds in LF and MF

Formulation	Peak area	Y= m x + c	Conc. In	% of marker
LF (piperine)	7652.6	y = 53.53x+ 22297	3.72463 gm/100gm	3.7
MF (piperine)	6481.57	y = 53.53x + 22297	3.15467 gm/100gm	3.15
LF (Zingiberine)	8427.02	y = 47.82x + 811	9.04068 gm/100gm	9.04
MF (Zingiberine)	9660.49	y = 47.82x + 811	10.3637 gm/100gm	10.36

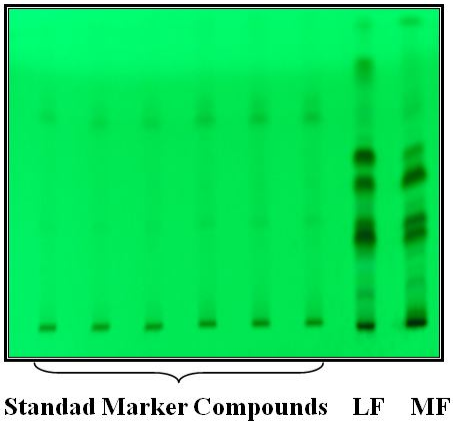
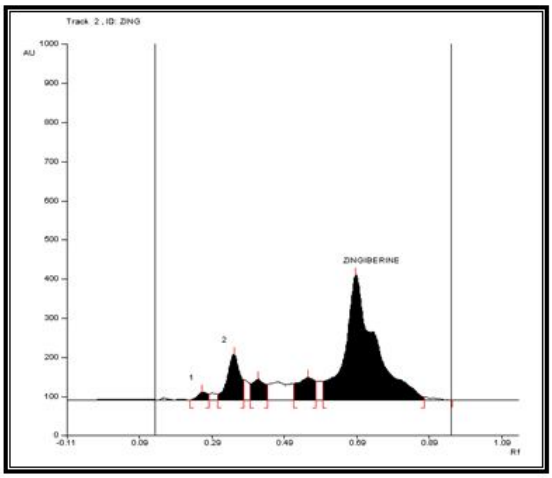


Figure 4. HPTLC plate for the Standard Zingeberene with LM and MF



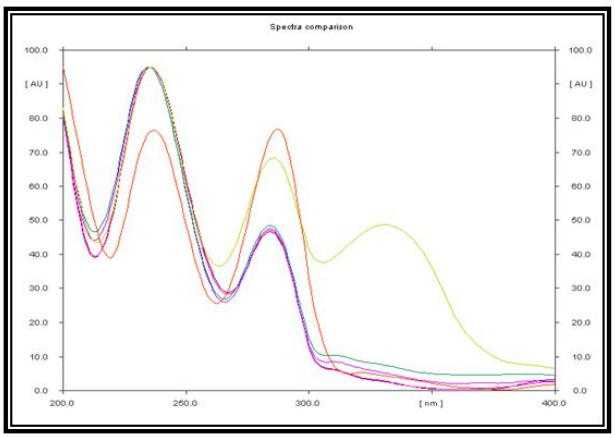


Figure 5. Densitometric Chromatogram of Zingeberene Standard

amount of constituents or are not standardized before formulating the market formulation. The quantity of zingiberine in LF was found to be less as compared to MF. This indicates that the MF contains more amount of ginger as compared to the quantity specified for its preparation.

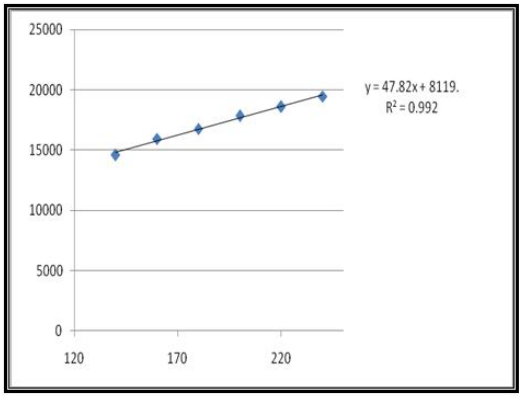


Figure 6. Calibration curve of Piperine

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