

HPTLC FINGERPRINTING IN THE STANDARDIZATION OF SULAHARAN YOGA: AN AYURVEDIC TABLET FORMULATION

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ABSTRACT

Quality assurance of herbal products may be ensured by proper quality control of the herbal ingredients and by means of good manufacturing practice. We have developed a simple scheme for the standardization and authentication of Sulaharan Yoga a poly herbal formulation using HPTLC. The present study signifies the use of TLC, HPTLC fingerprint profiles for deciding the identity, purity and strength of the polyherbal formulation and also for fixing standards for this Ayurvedic formulation.

Keywords: Standardization, HPTLC, Sulharan Yoga, Ayurvedic formulation.

INTRODUCTION

The Ayurveda, Unani and Sidha system of medicines have been in practice since thousands of years. The need of quality control for those medicines is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of Ayurvedic pharmacy during past era¹.

The concept of quality in those days was based on physical aspects of the plant materials such as identification, colour, odour, size, type, age, etc. Today there are additional requirements, distinct in nature, for modern routine quality control of botanical raw materials, in addition to physical tests and identification i.e. chemical composition. The Govt. of India has adopted the “fingerprint” approach² for botanicals because it supports the traditional concept and is easy to practice at different levels of sophistication. The British Pharmacopoeia³ has had an emphasis on using TLC and HPLC profiles to identify characteristic and active principles of herbal materials.

Sulaharan Yoga (SY), an Ayurvedic polyherbal formulation, consists of *Strychnos nux-vomica* and other seven ingredients in *Vati* (Tablet) form. It is one of the most widely used Ayurvedic product indicated for pain/colic, malabsorption syndrome, diarrhea, abdominal lump and digestive impairment.

Therefore, an attempt has been made to standardize Sulaharan Yoga, an Ayurvedic compound formulation based on their TLC and HPTLC fingerprint profile. The individual ingredients of Sulaharan Yoga, the in-house and marketed formulations were subjected to TLC and HPTLC fingerprinting along with superimposition with authentic markers.

MATERIALS AND METHODS

Chemicals and reagents:

All the chemicals used in the experiment were of analytical grade. Gallic acid, Gingerol, Piperine, Brucine, Strychine, Umbelliferone and Scopoletin were purchased from Sigma

Aldrich, USA. All the solvents used in the experiment were procured from Merck Specialties Pvt. Ltd, Mumbai, India.

Apparatus:

Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttentz, Switzerland)

Syringe: 100µL Hamilton (Bonadug, Switzerland)

TLC chamber: Glass twin trough chamber (20× 10× 4).

Densitometer: TLC scanner 3 with CATS software; CAMAG

HPTLC Plate: 20×10cm, 0.2 precoted with silica gel 60F₂₅₄; Merck

pH meter: Elico Ltd., Hyderabad, India.

Flame Photometer: Digital Biomed Flame Photometer, Hyderabad.

Muffle furnace: Dolphin Industries Ltd., Mumbai.

Plant materials:

Sulaharan Yoga consists of 1. *Terminalia chebula*(Combretaceae, dried fruit), 2. *Zingiber officinale* (Zingiberaceae, dried rhizome), 3. *Piper nigrum* (Piperaceae, dried fruit), 4. *Piper longum* (Piperaceae, dried fruit), 5. *Strychnos nux-vomica* (Fabaceae, dried seed), 6. *Ferula foetida* (oleo-gum-resin), 7. Sulphur and 8. Rock salt (Saindhava lavana). All these ingredients were procured from the local market of Jeypore, Koraput, Odissa, India and all the plant material were authenticated by Mr. S.R. Dash H.O.D Dept of Botany Vikram Dev College Jeypore, Koraput Odisa. Voucher specimens (JCP/Lab-6/8/2009) of the same have been deposited in the museum of Dept. of Pharmacognosy, Jeypore College of Pharmaceutical Sciences for future reference.

Preparation of sulaharan yoga:

In-house formulation of Sulaharan Yoga was prepared as per Ayurvedic Formulary of India. The ingredients number 1 to 4 were washed, dried and powdered and passed

through 80# sieve. The ingredients number 5, 6, 7 and 8 were cleaned and powdered individually and passed through 80# sieve. All the ingredients were mixed thoroughly in specified ratio (1 parts each) to obtain a homogeneous blend. The blended mass was expelled through tablet punch machine fitted with suitable die. The rolled vatis (Tablets) were dried in a tray-dryer at a temperature not exceeding 60°C. It was packed in a tightly closed glass containers for further use.

Marketed samples:

One marketed sample of Sulaharna Yoga (Sarmayu) were chosen for the study. Sarmayu and the in-house preparation were standardized based on their fingerprint profiles.

HPTLC finger printing profile:

HPTLC study^{4,5} of methanolic extracts of the individual ingredients, in-house formulation and marketed formulation were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in all the formulations. For HPTLC, 2gm of each sample were extracted with 25ml of methanol on boiling water bath for 25minutes consecutively three times using fresh portion of 25ml methanol, filtered and concentrated. The chromatograph was performed by spotting standards and extracted samples on pre coated silica gel aluminium plate 60F-254 (10cm×10cm with 250µm thickness) using Camag Linomat IV sample applicator and 100µl Hamilton syringe. The samples, in the form of bands of length 5mm, were spotted 15mm from the bottom, 10mm apart, at a constant application rate of 15nl/s using nitrogen aspirator. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance /reflectance mode.

RESULTS AND DISCUSSION

The well resolved TLC profile of the marker compounds corresponding to the individual ingredients of *Sulaharana yoga* was presented in (Fig 1a-e) to authenticate the presence of these ingredients in all the samples of *Sulaharana yoga*.

From superimposition study a band (R_f 0.44) corresponding to Gallic acid (fig 1a) is visible in both *Terminalia chebula* and *Sulaharana yoga* formulations, indicate the presence of *Terminalia chebula* in the formulations. Band (R_f 0.35) corresponding to Gingerols (blue spot) is visible in both the ingredient and formulations, indicate the presence of *Zingiber officinale* (fig 1b). Similarly bands with R_f values 0.50 corresponding to Piperine indicates the presence of both *Piper nigrum* (Fig 1c) and *Piper longum* (Fig 1d) in the formulations. Bands (R_f 0.15 and 0.33) corresponding to Brucine and strychnine is visible in both the ingredient and formulations indicate the presence of *Strychnos nuxvomica* (Fig 1e). A band (R_f 0.40) corresponding to Umbelliferone (Fig 1f) is visible in both the ingredient and formulations, indicate the presence of *Ferula foetida* in the formulations.

The TLC profiles of both the formulations are superimposable (Fig 2) indicating the presence of all the constituents in the marketed and in-house formulation.

HPTLC fingerprint profile of the Sulaharna Yoga formulations are depicted in figure 3a and 3b indicates the presence of all the ingredients in proportional quantity in the formulations without any impurity. This confirms the consistency in the preparation of the in-house and marketed product.

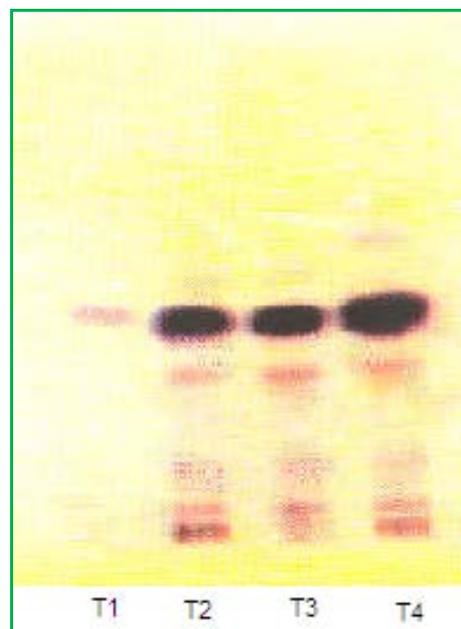


Figure 1a: TLC fingerprint profile of *Terminalia chebula*

Track 1: Gallic acid; Track 2: Sulaharana Yoga (In-house); Track 3: Sulaharana Yoga (Sharmayu); Track 4: *Terminalia chebula*; Solvent System: Chloroform: Ethyl acetate: Formic acid (2.5: 2: 0.8)

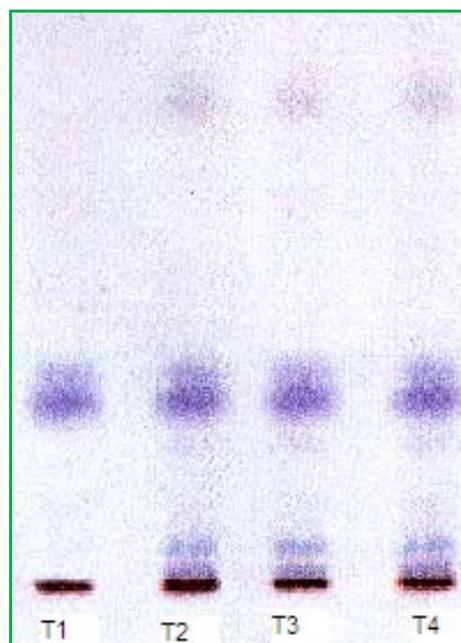


Figure 1b: TLC fingerprint profile of *Zingiber officinale*

Track 1: Gingerols; Track 2: Sulaharana Yoga (In-house); Track 3: Sulaharana Yoga (Sharmayu); Track 4: *Zingiber officinale*; Solvent System: n-Hexane: Ether (40: 60)

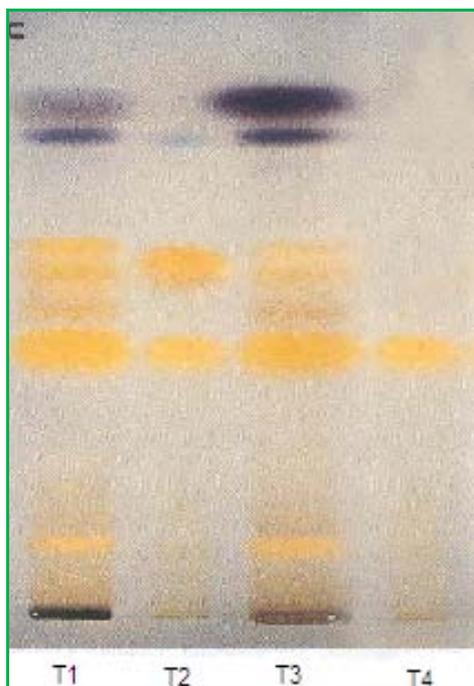


Figure 1c: TLC fingerprint profile of *Piper nigrum*
 Track 1: Sulaharana Yoga (In-house); Track 2: *Piper nigrum*; Track 3: Sulaharana Yoga (Sharmayu); Track 4: *Piperine*; Solvent System: Toluene: Diethyl ether: Dioxane (62.5: 21.5: 16).

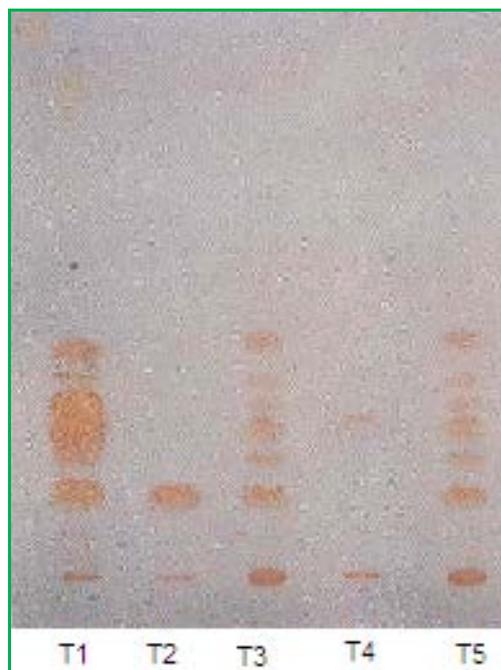


Figure 1e: TLC fingerprint profile of *Strychnos nuxvomica*
 Track 1: *Strychnos nuxvomica*; Track 2: Brucine; Track 3: Sulaharana Yoga (In-house); Track 4: *Strychine*; Track 5: Sulaharana Yoga (Sharmayu); Solvent System: Toluene: ethyl acetate: Diethylamine (70: 20: 10).

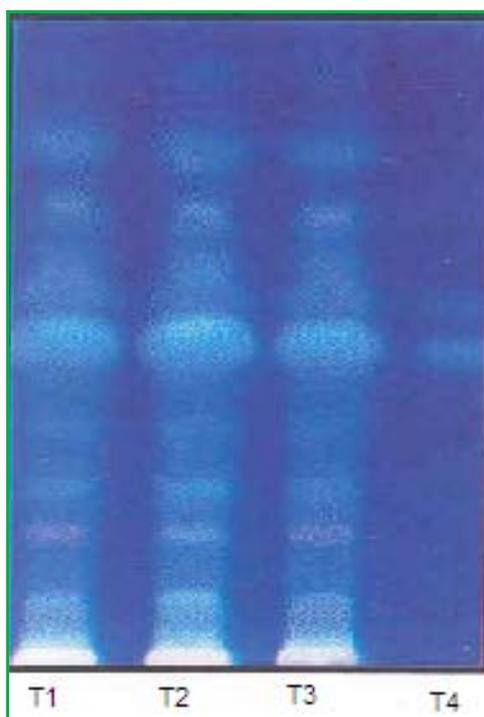


Figure 1d: TLC fingerprint profile of *Piper longum*
 Track 1: Sulaharana Yoga (In-house); Track 2: Sulaharana Yoga (Sharmayu); Track 3: *Piper longum*; Track 4: *Piperine*; Solvent System: Toluene: Diethyl ether: Dioxane(62.5: 21.5 : 16).



Figure 1f: TLC fingerprint profile of *Ferula foetida*
 Track 1: *Ferula foetida*; Track 2: Sulaharana Yoga (Sharmayu); Track 3: Sulaharana Yoga (In-house); Track 4: *Umbelliferone*; Solvent System: Toluene: ether (1:1 saturated with 10% acetic acid)

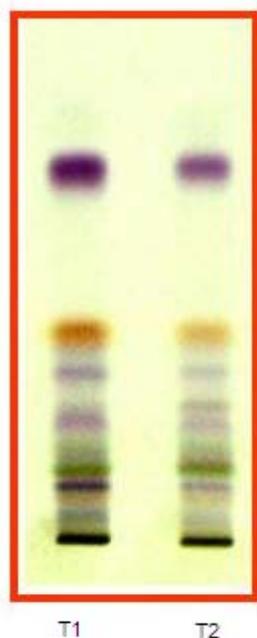


Figure 2: TLC fingerprint profile of formulations

Track 1: Sulaharana Yoga (In-house); Track 2: Sulaharana Yoga (Sharmayu); Solvent System: Toluene: ethyl acetate: formic acid (5:15:0.5)

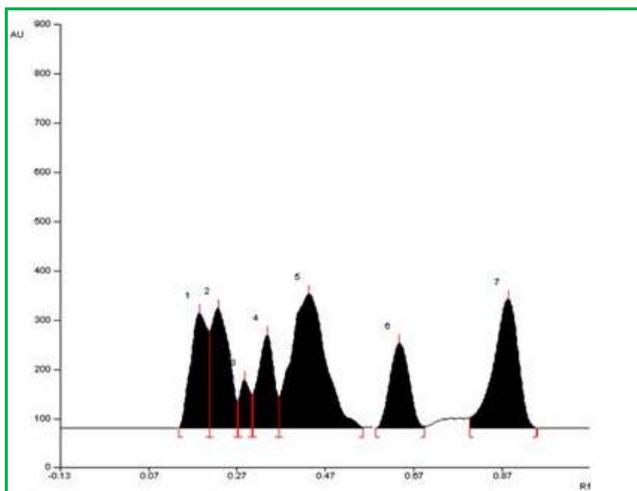


Figure 3a: HPTLC fingerprinting of sulaharan yoga

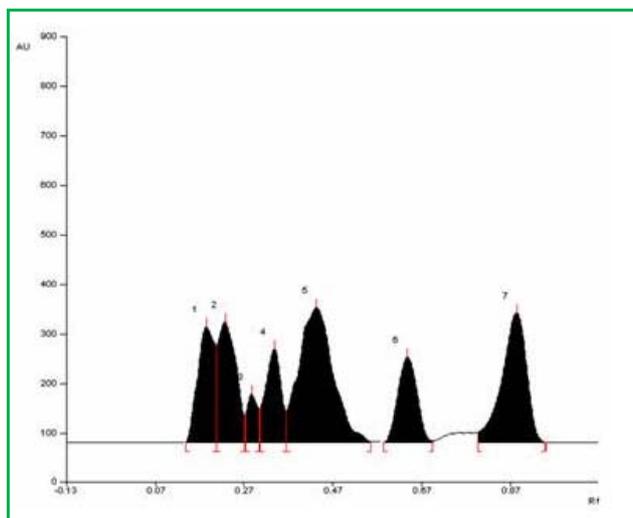


Figure 3b: HPTLC fingerprinting of sulaharan yoga

CONCLUSION

It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers ensures that the concentration and ratio of components in the herbal mixture are present in reproducible levels in raw materials and in the final dosage form. In this way use of markers and chromatographic fingerprinting can give information assisting manufacturing control and assuring batch to batch consistency.

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