AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

QHPTLC Method for the Estimation of *Santalum album* In Padoladi Ghritham- A Polyherbal Ayurvedic Formulation

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ABSTRACT

A Quantitative High Performance Thin Layer Chromatography (QHPTLC) method was developed for the estimation of *'Candana'* (*Santalum album*), one of the herbal ingredients present in the Ayurvedic polyherbal formulation '*Padoladi Ghritham'*. For this, both the methanol extracts of *'Candana'* crude drug and *Padoladi Ghritham* were run using a mobile phase Chloroform : Isopropyl alcohol : Toluene (8:1:1, v/v/v) on a silica G 60 F_{254} stationary phase. Methanol extract of *Candana* gave a spot with R_f value of 0.76. The methanol extract of *Padoladi Ghritham* also gave a spot with R_f value of 0.76. The chemical equivalency of these two spots was revealed by the same pattern of the UV spectrum with a λ_{max} of 290 nm. Then the estimation was done graphically by preparing Quarter Standard, Half Standard, Normal Standard, and Double Standard of the formulation. A calibration plot was drawn between the concentration of standards and peak area of the reference spots. The concentration of *Candana* in the samples was found from the corresponding AUC obtained for the reference peak of the samples. A regression coefficient of 0.989 was obtained. The developed QHPTLC method is a simple and accurate one and can be used as a tool for the quantification of each ingredient in a polyherbal formulation.

Keywords: QHPTLC, *Santalum album*, *Padoladi Ghritham*, AUC, Regression Coefficient.

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Received 20 January 2017, Accepted 29 January 2017

Please cite this article as: Presannakumaran PN *et al*., QHPTLC Method for the Estimation of *Santalum album* In Padoladi Ghritham- A Polyherbal Ayurvedic Formulation. American Journal of PharmTech Research 2017.

INTRODUCTION

Herbal medicines include herbal extracts, herbal drug preparations and herbal drugs. Herbs include crude plant material such as leaves, flowers, fruit, seed, stem, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal preparations include comminuted or powdered materials or extracts, tinctures and fatty oils of herbal materials, which may be produced by extraction, fractionation, purification, concentration or other physical or biological processes.[1] Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the product can be called "mixture herbal product" or "polyherbal formulation". Finished herbal products may contain excipients in addition to the active ingredients. However, finished products to which chemically defined active substances have been added, including synthetic compounds or isolated constituents from herbal materials, are not considered to be herbal.[2] Therefore if phytopharmaceuticals have to be regarded as drugs, they should be standardized and the quality validated. Ayurvedic formulations are polyherbal preparations and the quality parameters prescribed for polyherbal phytopharmaceuticals are equally applicable to them also.[3]

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definite qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process.[4,5]

Several problems not applicable to synthetic drugs often influence the quality of herbal drugs.[6,7] For instance:

1. Herbal drugs are usually mixtures of many constituents.
2. The active principles in most cases are unknown.
3. Selective analytical methods or reference compounds may not be available commercially.
4. Plant materials are chemically and naturally variable.
5. The source and quality of the raw material are variable.

Indian Scenario

The necessity of standardization of Ayurvedic products is immensely felt and the Department of AYUSH has taken initiatives in this direction. The first step was to standardize crude drugs used in Ayurvedic formulations. Now majority of the crude drugs used in Ayurveda got standardized.

These are available as monographs in the various volumes of Ayurvedic Pharmacopoeia of India (API). Similarly majority of the conventional Ayurvedic formulations also got standardized. Now in the official monograph, one can find the composition of the Ayurvedic product, its method of preparation and various standards for the quality control of the product. Thus uniformity is brought among products specified in the official monograph which helps in the control over the production and sale of Ayurvedic products.

Though Ayurvedic Pharmacopoeia of India have set standards for crude drugs and formulations, serious limitations are felt especially in the case of formulations. The official monograph quite often prescribes limits to the bulk property of the formulations such as specific gravity, optical rotation, refractive index and chemical constants such as acid value, saponification value, iodine value, peroxide value, ester value etc.[8,9] With these parameters it is impossible to ascertain precisely the quantity of a crude drug present in a formulation. In a very few formulations certain markers are identified and quantified. Although such a step is acceptable to the scientific community, it is confined to very few crude drugs and formulations only.[10, 11]

Presently it is very difficult, rather impossible to determine exactly the amount of crude drug present in an Ayurvedic formulation. Many Ayurvedic formulations in the market do not contain all the ingredients they should contain. If at all, all the ingredients are present in a formulation each one may not be present to the required amount. This situation is alarming, and there should evolve very specific methods for the accurate quantification of each ingredient present in a formulation. QHPTLC is a method developed in this line and is found to be an effective tool in addressing the above limitation.[11,12]

MATERIALS AND METHOD

Chemicals

Chloroform, Isopropyl alcohol, Toluene and Methanol. All chemicals used were HPLC grade and purchased from Merck Specialties Mumbai

Crude Drugs

Trichosanthus dioica, Azadirachta indica, Picrorhiza kurroa, Coscinium fenestratum, Vetiveria zizanioides, Terminalia chebula, Terminalia belerica, Eblica officinalis, Adhatoda zeylanica, Tragia involucrata, Bacopa monnieri, Oldenlandia corymbosa, Cyperus rotundus, Solanum indicum, Glycyrrhiza glabra, Holarrhena antidysenterica, Coleus vettiveroides, Santalum album, Piper longum, Cow's ghee.

All the drugs except the sandal wood, were procured from a crude drug shop at Karunagapally, Kollam District, Kerala State, India. A genuine sample of *Santalum album* (Sandal wood or '*Candana')* was purchased from the "*Vanasree"* outlet of Forest Department, Government of Kerala.

Apparatus

CAMAG Linomat IV Autosampler, CAMAG TLC Scanner-III, UV Cabinet, TLC Plate Heater, 100µl Syringe (Hamilton Switzerland), Twin Trough Chamber, Silica Gel $60F_{254}$ Plates (Merck), Soxhlet Apparatus.

Methodology

The QHPTLC method for the quantification of *'Candana'* (*Santalum album*) in the Ayurvedic formulation *Padotadhi Ghritham* is undertaken in the present study. The drug *Candana'* (*Santalum album*) is official in Ayurvedic Pharmacopoeia of India, Part-I, Volume-III[13].

Preparation of Methanol extract of crude drug

25 g of the coarse powder of authenticated sample of *Candana'* (*Santalum album*) was packed in a thimble made of filter paper. The thimble was inserted into a Soxhlet extractor and extracted with 50 ml of methanol, 5 cycles a day for 3 days. The volume was reduced by evaporation and made up to 10 ml.

Preparation of standard formulation

The Ayurvedic formulation *Padotadhi Ghritham* is originally described in *Astangahridaya*.[14] A standard preparation of the Ayurvedic formulation was made strictly as per the procedures described in the Ayurvedic Pharmacopoeia of India.[15] Official formula for preparation of *Padotadhi Ghritham* according to API is given in (Table 1). Three standard preparations namely 'Quarter Standard (Q-Std)', 'Half Standard (H-Std)' and 'Double Standard (D-Std)' were also prepared by taking quarter, half and double the quantities of individual ingredients present in the Standard formulation.

Preparation of methanol extracts of standard and samples

Four marketed samples of *Padoladhi Ghritham* manufactured by four different manufacturers were purchased from the local market. Methanol extracts of the four standards and the four samples were prepared from 25 g each of the standards and samples by solvent extraction using 50 ml of methanol by refluxing on a boiling water bath for 3 hours. The volume was reduced by simple evaporation and made up to 10 ml.

Table 1.Formula for the preparation of 768g of *Padoladhi Ghritham*[14,15]

Sl. No.	Sanskrit Name	Plant part *	Malayalam name	Botanical name	Qty.
KVATHA					
1	Patola	Pl.	Padavalam	*Trichosanthus dioica*	48 g
2	Nimba	St. Bk.	Veppu	*Azadirachta indica*	48 g
3	Katuka	Rt/Rz.	Katukurohini	*Picrorhiza kurroa*	48 g
4	Darvi	St.	Maramanjal	*Coscinium fenestratum*	48 g
5	Sevya	Rt.	Ramacham	*Vetiveria zizanioides*	48 g
6	Haritaki	P.	Kadukka	*Terminalia chebula*	48 g
7	Bibhitaki	P.	Thannikka	*Terminalia belerica*	48 g
8	Amalaki	P.	Nellikka	*Emblica officinalis*	48 g
9	Vasa	Rt.	Adalodakam	*Adhatoda zeylanica*	48 g
10	Dhanvayasa	Pl.	Kodithoova	*Tragia involucrate*	48 g
11	Trayanti	Pl.	Brahmi	*Bacopa monnieri*	48 g
12	Parpata	Pl.	Parpatakapullu	*Oldenlandia corymbosa*	48 g
13	Amalaki	P.	Nellikka	*Emblica officinalis*	768 g
14	Water for decoction Volume reduced to				12.288 L 3.072 L
15	Goghritha	-	Pasuvin Neyyu	*Cow's Ghee*	768 g
KALKA					
16	Musta	Rz.	Muthanga	*Cyperus rotundus*	24 g
17	Bhunimba/Brhati▪	Rt.	Putharichunda	*Solanum indicum*	24 g
18	Yasti	Rt.	Irattimadhuram	*Glycyrrhiza glabra*	24 g
19	Kutaja(Indrayava)	Sd.	Kutakappalayari	*Holarrhena antidysenterica*	24 g
20	Udicya	Rt.	Iruveli	*Coleus vettiveroides*	24 g
21	Candana	Ht. Wd	Chandanam	*Santalum album*	24 g
22	Pippali	Fr.	Thrippali	*Piper longum*	24 g

* Pl: Whole Plant, P: Pericarp, St. Bk: Stem Bark, St: Stem, Rt: Root, Rz: Rhizome, Sd: Seed, Ht. Wd: Heart Wood and Fr: Fruit

As per the Ayurvedic Pharmacopoeia of India, the drug *Bhunimba* is *'Kiriyath',* the botanical name of which is *Andrographis paniculata.* In North India this drug is used for *Bhunimba*, but in Kerala *'Putharichunda'* is used instead. The Sanskrit name of *'Putharichunda'* is *Brhati* and its botanical name is *Solanum indicum.* For the present research work *Solanum indicum* is used. Its use in the formulation is authorized by The Pharmacopoeia (Malayalam) published by the Kerala State Ayurvedic Publications, Trivandrum, 1996.[16]

HPTLC Chromatogram development and scanning

The chromatograms were developed on 10 x 20 cm HPTLC Silica gel $60F_{254}$ plates previously activated by heating at 110^{o}C on a TLC Plate Heater for 15 minutes. On to this plate 2µl of the extracts were spotted in the following order using the applicator Linomat-IV of CAMAG.

Table 2.Number of Tracks and Track identity

Track No.	Track identity
Track- 1	Methanol extract of *Candana* API *(Santalum album)*
Track- 2	Methanol extract of Q-Std
Track- 3	Methanol extract of H-Std
Track- 4	Methanol extract of N-Std
Track- 5	Methanol extract of D-Std
Track- 6	Methanol extract of Sample-A
Track- 7	Methanol extract of Sample-B
Track- 8	Methanol extract of Sample-C
Track- 9	Methanol extract of Sample-D

The plate was developed in a Twin Trough chamber using Chloroform: Isopropyl alcohol: Toluene :: 8 : 1 : 1 (v/v/v). The solvent front was allowed to run about 9 cm. The plate was taken out, dried and visualized under the UV cabinet. The photograph of such a chromatogram is shown in (Figure1). Each track on the plate was scanned by CAMAG TLC Scanner. The same procedure was done in triplicate.

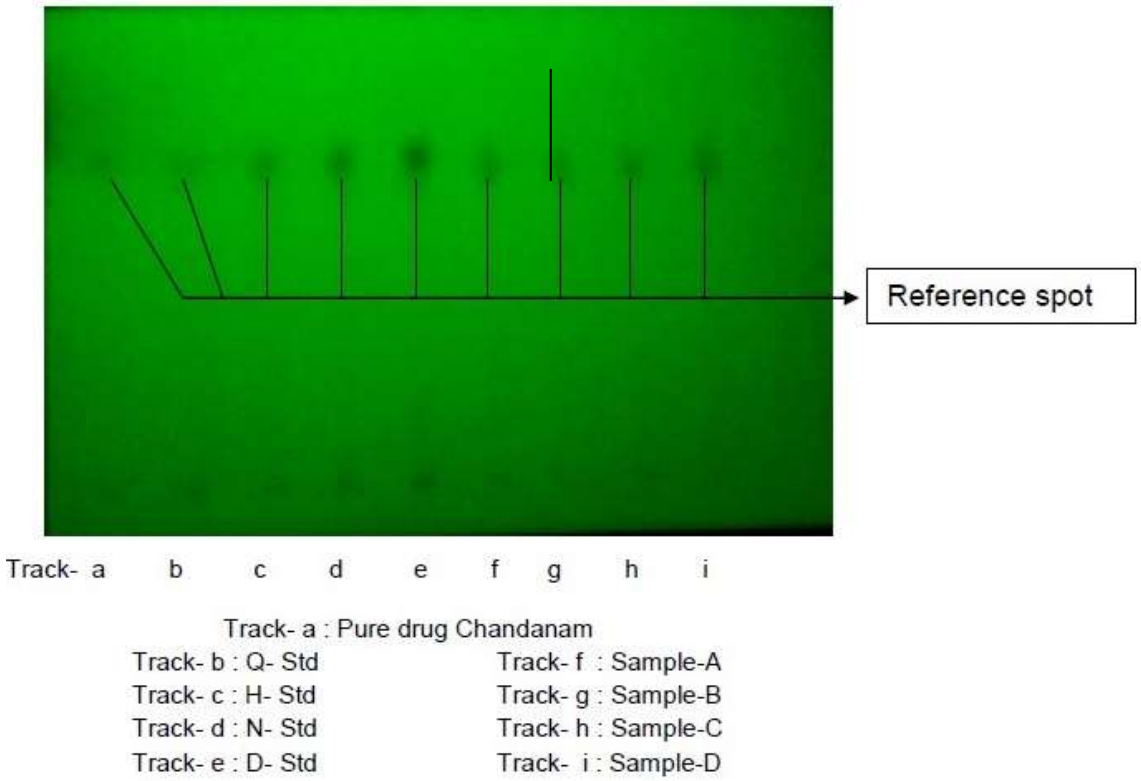


Figure 1: Photograph of the QHPTLC plate for *Candana*

The reference spot on each track with R_f value of 0.76 was integrated to get the AUC. The chromatogram of the plant extract, the four standards and the four samples are shown below.

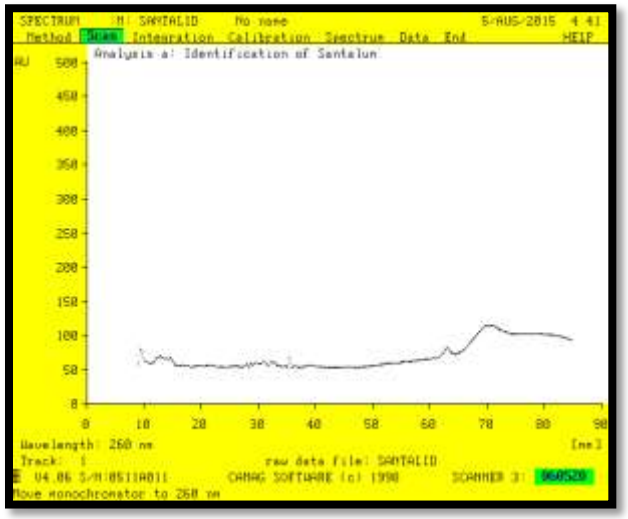


Figure 2(a.1)- Chromatogram of plant extract of Candana

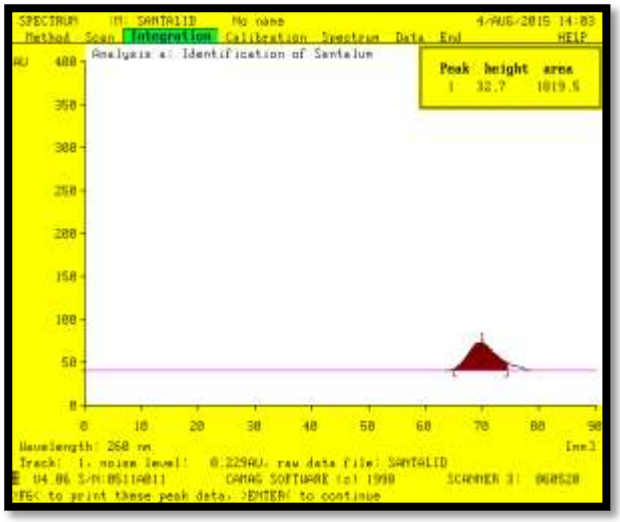


Figure 2(a.2)- Integrated chromatogram

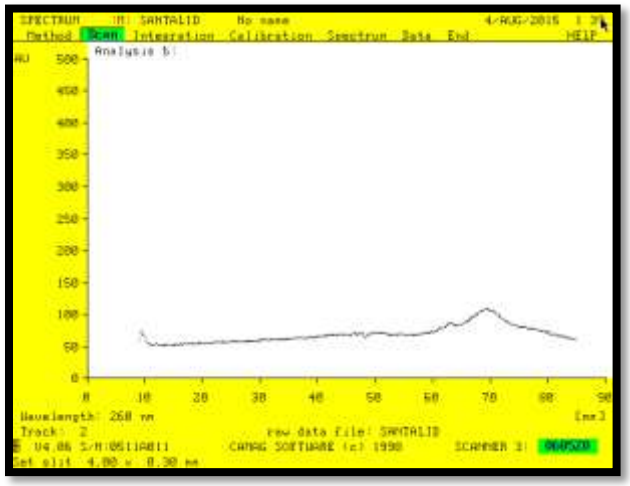


Figure 2(b.1)- Chromatogram of Quarter standard

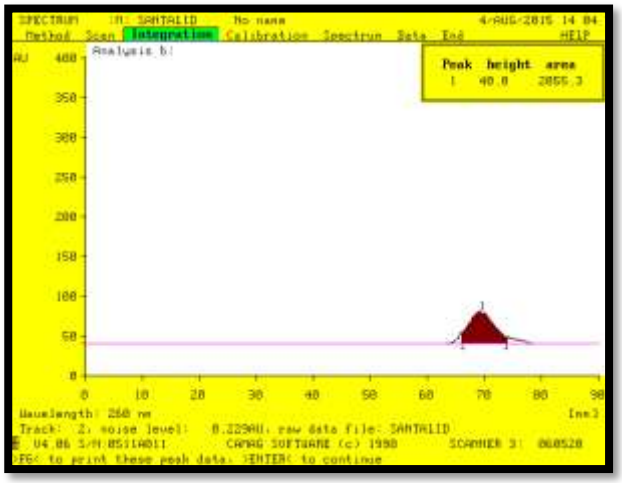


Figure 2(b.2)- Integrated chromatogram

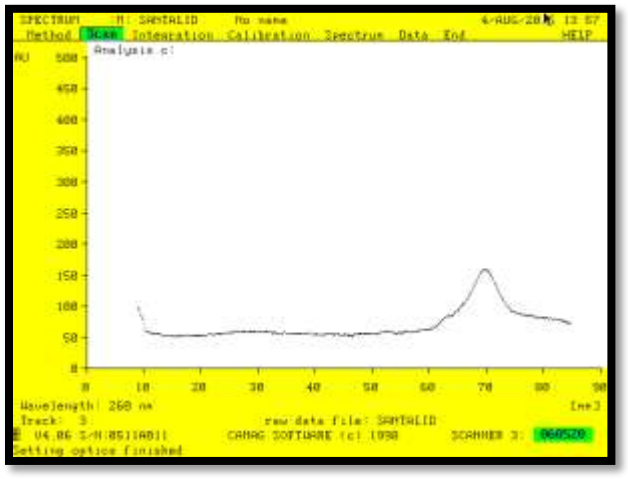


Figure 2(c.1)- Chromatogram of Half standard

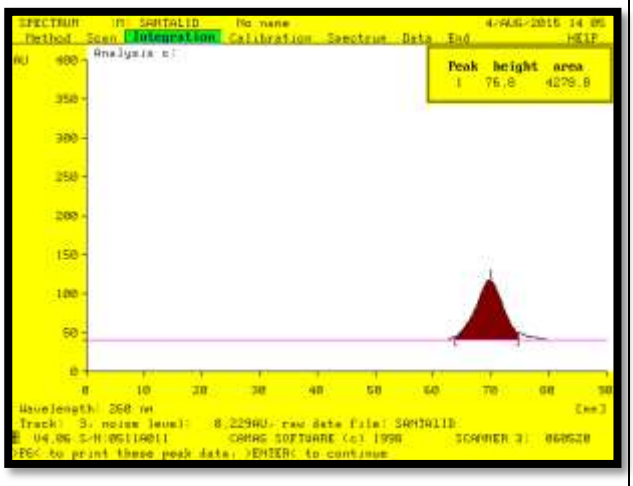


Figure 2(c.2)- Integrated Chromatogram

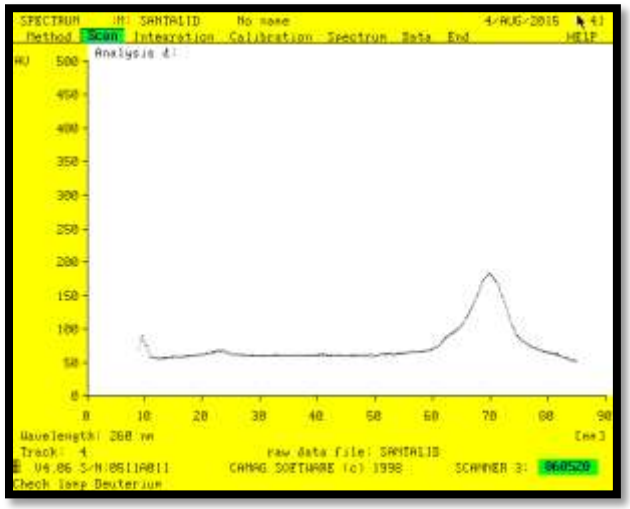


Figure 2(d.1)- Chromatogram of Normal standard

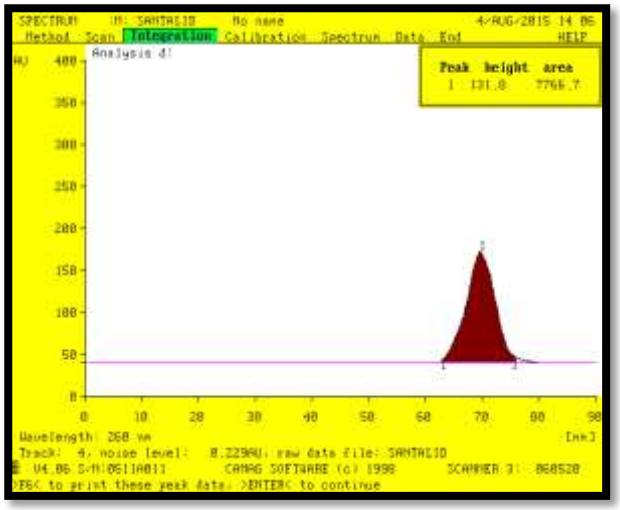


Figure 2(d.2)- Integrated chromatogram

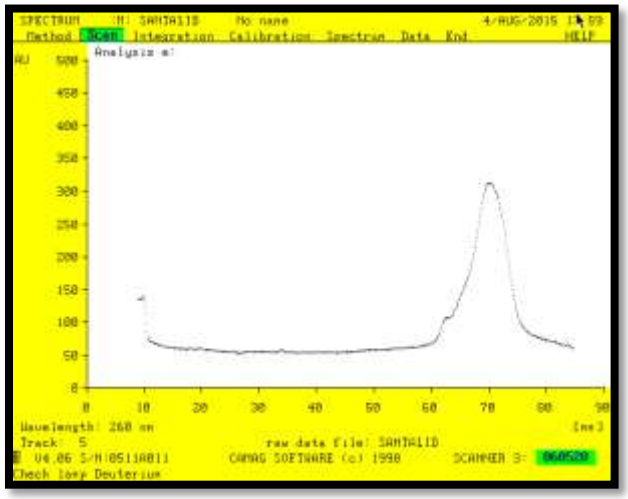


Figure 2(e.1)- Chromatogram of Double standard

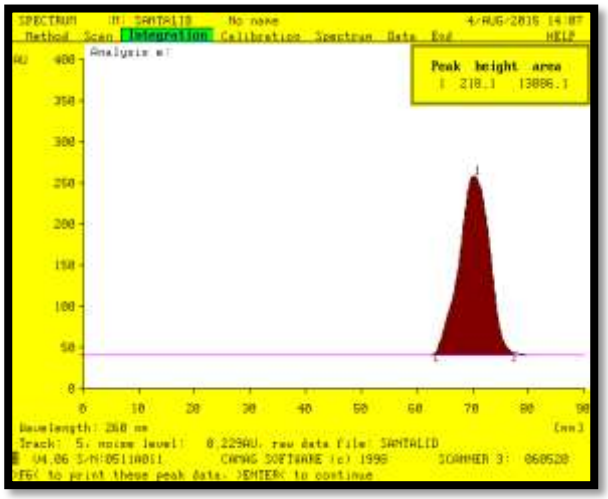


Figure 2(e.2)- Integrated chromatogram

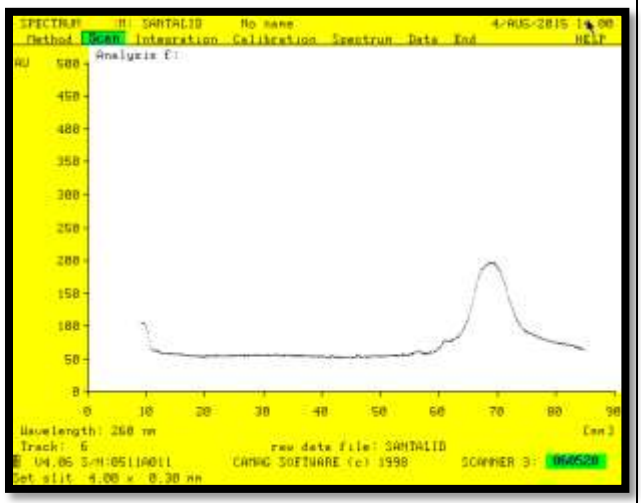


Figure 2(f.1)- Chromatogram of Sample A

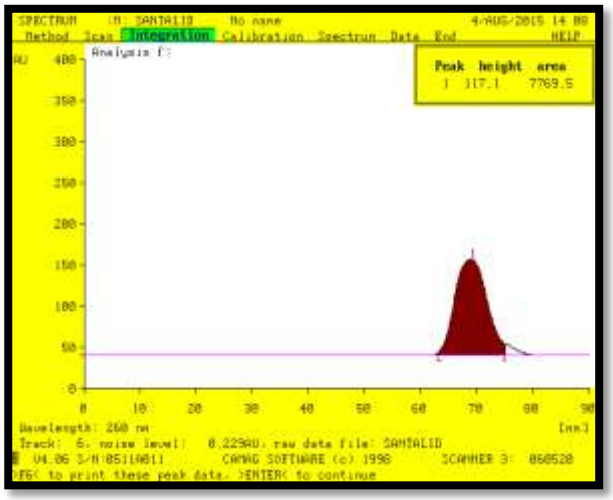


Figure 2(f.2)- Integrated chromatogram

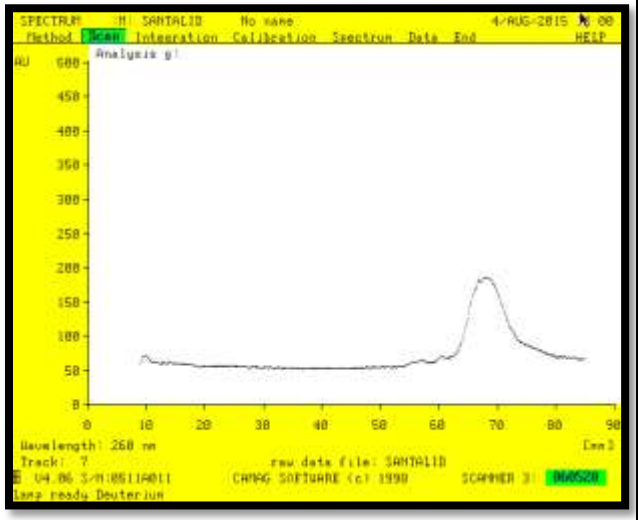


Figure 2(g.1)- Chromatogram of Sample B

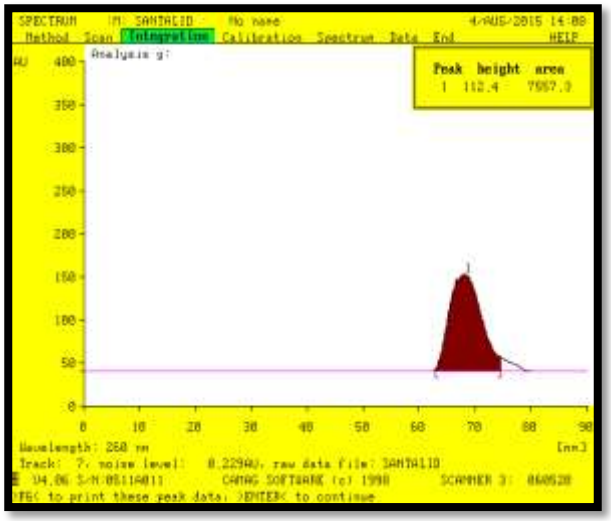


Figure 2(g.2)- Integrated chromatogram

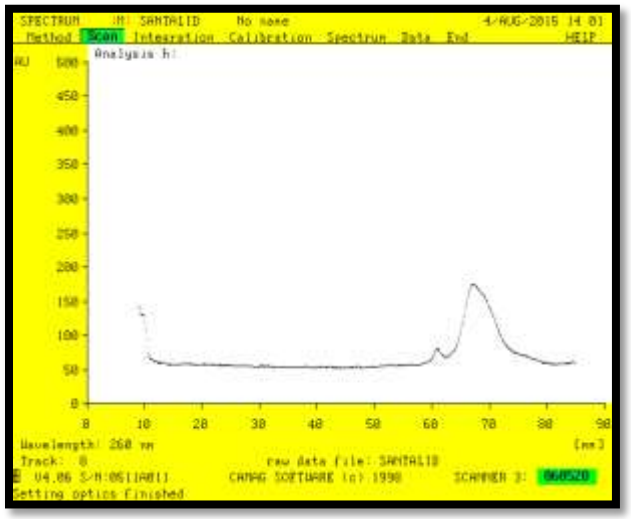


Figure 2(h.1)- Chromatogram of Sample C

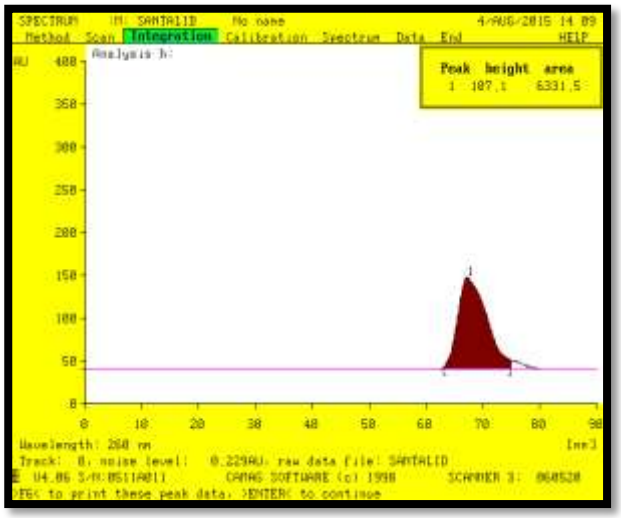


Figure 2(h.2)- Integrated chromatogram

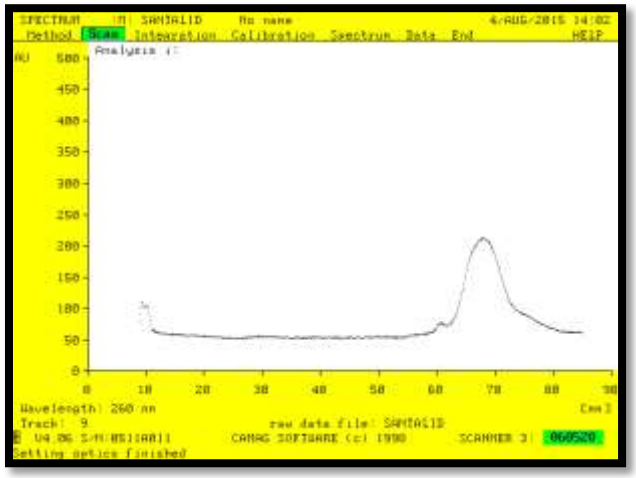


Figure 2(i.1)- Chromatogram of Sample D

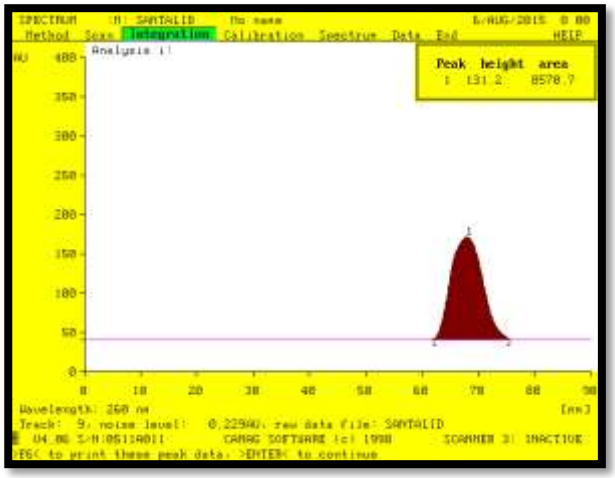
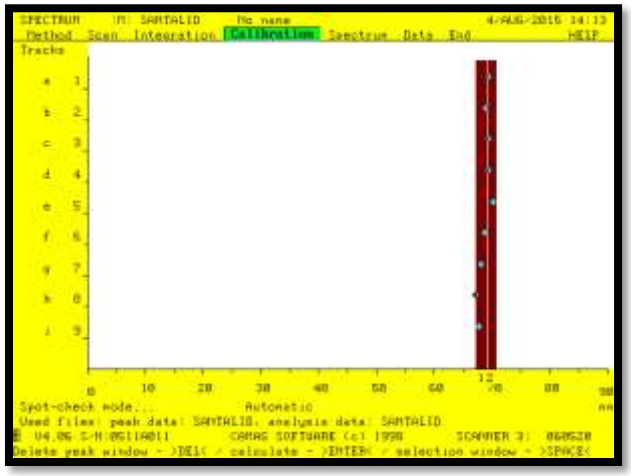


Figure 2(i.2)- Integrated chromatogram



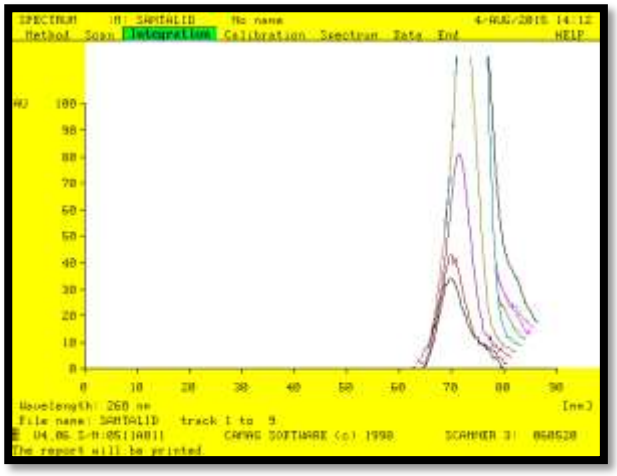
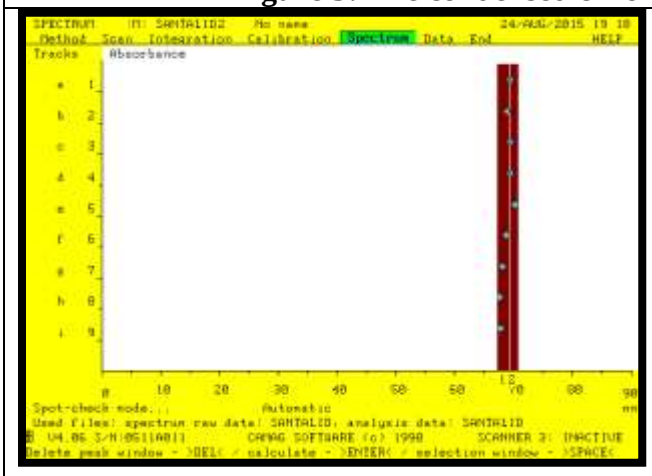


Figure 3: The condensed chromatogram of Trial-1 (Candana)



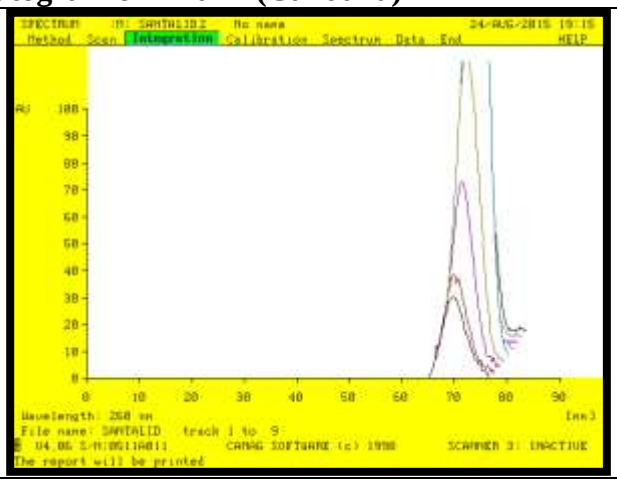
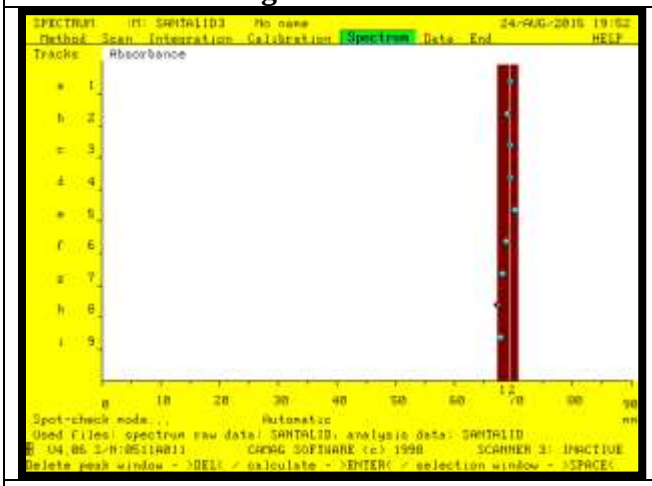


Figure 4: The condensed chromatogram of Trial-2 (Candana)



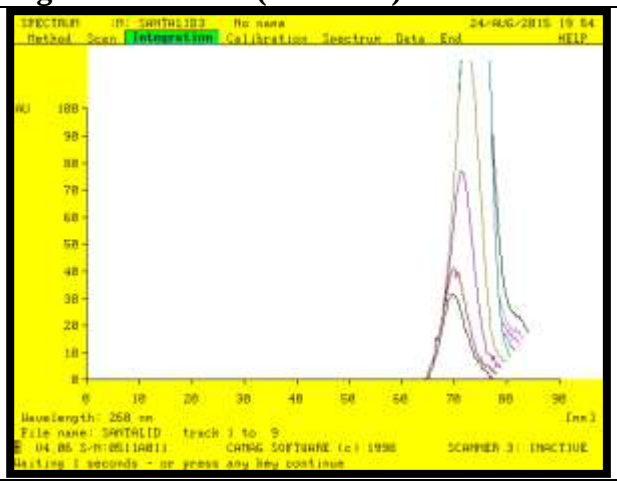


Figure 5: The condensed chromatogram of Trial- 3 (Candana)

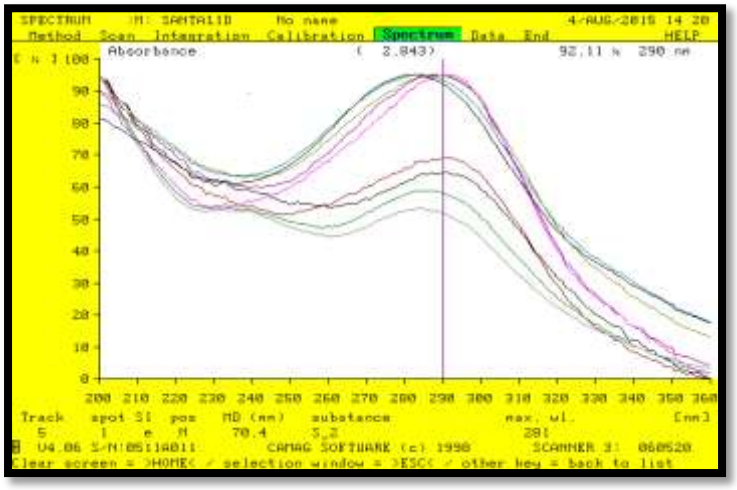


Figure 6: UV Spectrum of the Reference Spot on all the tracks

RESULTS AND DISSCUSSION

Quantitative estimation of one of the herbal ingredients *Candana* present in the polyherbal formulation *Padoladhi Ghrutham* was carried out by QHPTLC method. For the optimization of the method, different mobile phase compositions were used to get a good separation. Among the various mobile phases tried, Chloroform: Isopropyl alcohol: Toluene:: (8 : 1 : 1 v/v/v) showed a good resolution. The methanol extract of the crude drug *Candana*, methanol extracts of standard preparation of *Padoladhi Ghrutham* and the methanol extracts of the marketed samples of *Patoladhi Ghrutham*, gave dark brown spots with an R_f value of 0.76 when observed through the UV cabinet. The photograph of developed HPTLC plate is given in (Figure1). The chemical equivalency of the spots was confirmed by the UV surface scanning. The overlaid UV spectrum of the spots is given in (Figure 6). The spectra had a λ_{max} of 290 nm.

Table 3: AUC of each spot obtained in the QHPTLC analysis of *Candana*

Sl. No.	Track identity	AUC of the peak			Mean Value ± SD
		Trial-1	Trial-2	Trial-3	
1	Track-1: Methanol extract of *Candana*	1819.5	1809.0	1757.7	1795.40±33.07
2	Track-2: Methanol extract of Q- Std.	2055.3	1975.0	2067.3	2032.53±50.19
3	Track-3: Methanol extract of H- Std.	4278.8	4281.2	4310.4	4290.13±17.59
4	Track-4: Methanol extract of N-Std.	7766.7	7898.4	7949.5	7871.53±94.32
5	Track-5: Methanol extract of D- Std.	13886.1	12684.8	13093.4	13221.43±610.80
6	Track-6: Methanol extract of Sample A	7769.5	7895.7	7743.4	7802.86±81.45
7	Track-7: Methanol extract of Sample B	7557.3	7516.9	7493.8	7522.66±32.14
8	Track-8: Methanol extract of Sample C	6331.5	6254.8	6223.7	6270.00±55.48
9	Track-9: Methanol extract of Sample D	8570.7	8325.5	8119.9	8338.70±225.69

Table 4: Concentration vs AUC of Chandanam

Concentration (Mass units)	AUC (Average of 3 values)
1	2032.53
2	4290.13
4	7871.53
8	13221.43

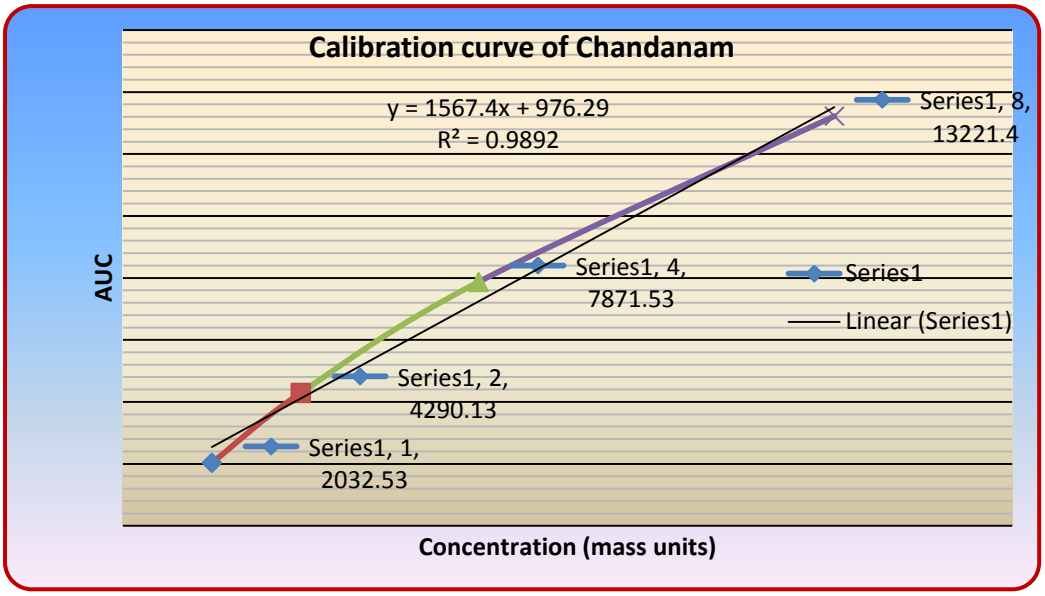


Figure 7: Calibration Plot of *Candana*

The AUC obtained for reference spots of the four standard tracks were used to plot a graph to establish the linearity between the concentration and AUC (Figure 7). The correlation coefficient obtained was within the acceptable limits. Thus it could be possible to obtain the quantities of the drug in question used in the different samples. From the graph the concentration of *Candana* present in the four marketed samples of the formulation namely Sample-A, B, C and D (Table 4) were found out.

Table 4: Concentration of *Candana* estimated in various marketed formulations by QHPTLC method

Sample	AUC (Average of 3 values)	Theoretical Concentration (Mass units)	Practical Concentration (Mass units)	Percentage label claim of *Candana* in the formulation (% w/w)
Sample-A	7802.86	4	4.36	109.00
Sample-B	7522.66	4	4.18	104.50
Sample-C	6270.00	4	3.38	84.50
Sample-D	8338.70	4	4.70	117.50

The percentage of drug present in the four marketed samples ranged from 84.50 to 117.50. The variation though very wide is expected from a biological system because of the inherent

variability. In the case of allopathic formulations the limit of assay is usually in the range of ± 5 to 10 %. In the case of polyherbal formulations, taking in to account the inconsistency and complexity of the biological system a wider range of ± 20 to 30 % may be prescribed. It is possible to achieve better range by a careful and rigorous selection of ingredients and adhering to Good Manufacturing Practices.

CONCLUSION

The QHPTLC technique is a very scientific method for the quantification of individual ingredients used in polyherbal formulations. The HPTLC fingerprinting can be used as a reliable method for the identification of individual crude drugs in a formulation. Having established official standards for the crude drugs and Ayurvedic formulations, it now becomes possible to fix a range for all the ingredients present in a formulation. The QHPTLC method described for *Padoladi Ghritham* can be extended to other type of formulations with suitable modifications. For example, in the case of liquid oral preparations, a Chloroform extract may be prepared instead of the methanol preparation.

ACKNOWLEDGEMENT

We acknowledge our sincere gratitude to the Principal, Govt. Medical College, Thiruvananthapuram and the Prof. & Head of Pharmacy for the help rendered to us in the completion of this research work. We place it on records the help rendered by Dr.G. Gopalakrishnan, SG Pharmacy, KP Road, Kayamkulam, KERALA, in preparing the standard formulation of *Padoladi Ghritham* utilizing the facilities available with him.

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