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Quantitative Determination of β-Sitosterol in *Solanum xanthocarpum* Fruits by a Validated HPTLC Method

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Abstract

Solanum xanthocarpum is an important medicinal plant belonging to the family Solanaceae. A simple, rapid, sensitive, accurate, repeatable and robust HPTLC method has been developed and validated for the estimation of β- sitosterol in the leaf extract of *Solanum xanthocarpum.* β- sitosterol was estimated at 206 nm by densitometry using Silica gel 60 F_{254} as stationary phase and Toluene: Ethyl acetate (7:3), and as mobile phase. Linearity was observed in the concentration range 20-120ng/spot for β-sitosterol. The limit of detection and limit of quantification were found to be 0.07 & 8.17ng/spot respectively for β-sitosterol. Developed method was validated according to the ICH guidelines with respect to precision, accuracy, specificity and robustness. The technique has been applied *for* the first time for the estimation of β-sitosterol in fruit extracts of *Solanum xanthocarpum*. Statical analysis data indicate the accuracy and reliability of the method.

Keywords

Solanum xanthocarpum; β-sitosterol; Herbal analysis Validation, Kantakari, ICHguidelines

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1.INTRODUCTION

Nature has blessed mankind with a treasure of medicinal plants. Natural products have always remained a profile source for the discovery of new drugs and are used since Vedic period [1]. *Solanum xanthocarpum* (Solanaceae) it comprises 90 genera and 2000-3000 species commonly known as Indian night shade or yellow berried night shade. found though in India mostly in dry places as a weed along roadside and waste lands. A very prickly bright green perennial herb, somewhat woody at base stem is zigzag, berries are green, leaves are ovate stellate hairy on both sides. Flowers are purple in colour [2]. In ancient Ayurveda plant is describes as pungent, bitter, digestive, alternative astringent. Root decoction used as febrifuge, effective diuretic and expectorant [3]. Charaka and Sushrutha used the extract of entire plant and fruits in internal prescription for bronchial asthma, tympanitis, piles, dysuria and for rejuvenation. Kantakari ghrita of Charaka is specific for cough and asthma [4]. Past of leaves is applied to relieve pain; Seeds act as an expectorant in cough and asthma; It is also used as throat infection and other inflammatory problems [5]. It is used as an antispasmodic cardiotonic, hypotensive and cytotoxic activities [6]. This plant has reported various phytoconstituents such as glycoalkoids, solanocarpine, solasodine, galactoside of β-sitosterol, methyester of 3,4-dihydroxycinnamic acid, isochlorogenic, neochlorogenic, chlorogenic acids (fruits) [7]. β-sitosterol is a dietary supplement, found in a variety of plant and plant oils. Phytosterols are similar in structure to cholesterol except some structural differences [8]. β-sitosterol is known for curing heart diseases and fighting high cholesterol levels in the body [9]. It is also known to be helpful in

prevention of different types of allergies, cancer, influenza, asthma, hair loss, migraine [10].

HPTLC method is used not only for quantification of phytochemicals [11] and qualitative analysis of plant extracts [12], but also for quality control of raw materials and standardization of polyherbal formulations [13], Literature survey reveals that there are no reports on HPTLC quantification of β-sitosterol from the leaves of *Solanum xanthocarpum.*

2.MATERIALS AND METHODS

2.1 Instrumentation and Chromatographic Conditions

The method was developed on Camag HPTLC system consisting of Linomat V semi-automatic applicator (Camag, Muttenz, Switzerland), Camag TLC scanner 3, equipped with win CATS software (version 1.4.3) and Camag syringe of 100µl capacity. Separation and identification of β-sitosterol were performed on aluminum silica gel 60 F_{254} TLC plates (10×10)cm, layer thickness 0.3mm (E-Merk).The samples and standard were applied on the 6mm width bands with constant application rate 150 $nL.s^{-1}$,with semi-automatic applicator under a flow of nitrogen gas(N_2),the space between the two spots was 6mm.Linear ascending development was carried out in Camag twin trough chamber. The chamber was prostrated with 20ml of mobile phase Chloroform: Toluene (9.5:0.5) for 20 min at room temperature (25ºC×2ºC) before insertion of the plate into mobile phase. The developed distance was 95.0 mm. After development the plates were dried. Dentiometric scanning at 206 nm was selected, the absorption maximum of spot, performed with Camag TLC Scanner 3 in reflectance –absorbance mode using slit dimension 6.00×0.4 mm, data resolution 100 $mm \cdot step^{-1}$, scanning speed $20mm \cdot sec^{-1}$ and baseline correction was used. The radiation source used was Deutrium lamp meeting a continuous radiation for UV-Visible region 190-800nm.The method was validated according to the ICH guidelines [14].

2.2 Chemicals and Standards

β-sitosterol was purchased from Yucca Enterprises, Mumbai. All other solvents, reagents and Silica gel 60 F_{254} precoated HPTLC plates (10×10cm) were purchased from Merk (Germany).

2.3. Plant Material

The leaves of *Solanum xanthocarpum* were collected from Warangal and were authenticated by Prof. Md. Taxonomist, Departement of Botany, Kakatiya University, Warangal, Telangana, India.

2.4. Preparation of Standard solution

Standard solution prepared by dissolving 1mg of β-Sitosterol in 1ml chloroform. Standard graph was plotted in the concentrations of 0.2-1.2µl solution apply to HPTLC plate to get range 20-120 ng/spot.

2.5. Preparation of the Samples drug extract

100 grams of *Solanum xanthocarpum* fruits were macerated in 100ml of methanol in 250 ml conical flask and kept for 1 day while shaking at regular intervals. Later the contents were filtered through whatmann No.41 paper. The extract were concentrated using rotary evaporator. From this 20mg leaf extract was dissolved in 1 ml chloroform to obtain concentration 1000µg/ml.

2.6. Validation of HPTLC Densitometry Method

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

2.6.1. Linearity

From standard solution of β-sitosterol different volumes were loaded on to TLC plate using semi-automatic applicator to obtain concentrations of 20,40.60,80,100 and120 ng/spot. Each different concentrations were applied 3 times on the plate. The plate was then developed using the mobile phase and the peak areas were plotted against the standard concentrations to obtain the calibration plot.

2.6.2. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the standard deviation and slope of the calibration curve.

2.6.3. Specificity

Specificity of the method was analyzed by comparing the standard and extract samples. The spot for β-Sitosterol in the sample was confirmed by comparing the R_f values of the sample spot with that of standard.

2.6.4. Precision

Precision of the method was verified in terms repeatability and intermediate precision. Repeatability(%RSD) was determined by analyzing β-Sitosterol standard solutions in the range (20,80,120 ng/spot) three times on the same day. Intermediate precision (%RSD) was assessed by analyzing these solutions (20,80,120ng/spot) on the three different days over a period of one week.

2.6.5. Accuracy

Accuracy of the method was established by performing recovery experiments using the standard addition method. To the pre analyzed samples of the plant leaf extract, standard β-Sitosterol solution was added by spiking at three different levels (80%,100% and 120%) and the mixture was analyzed by the proposed method.

2.6.6. Robustness

By introducing small changes in the mobile phase composition, mobile phase volume and duration of mobile phase saturation the effects on the results were examined. Robustness of the method was done in triplicate in a concentration level of 80ng/spot and the %RSD of peak area was calculated.

3.RESULTS AND DISCUSSION

The mobile phase Toluene: Ethyl acetate (7:3) gave good resolution for β-Sitosterol peak at R_f =0.29 when the chamber was saturated with mobile phase for 20 min at room temperature (25ºC±2ºC) during HPTLC determination of β-Sitosterol from plant fruit extract. The plate was visualized under UV light at 206 nm with derivatization. Identity of β-Sitosterol bands in sample chromatograms was confirmed by the comparison of chromatogram and UV spectra obtained from the sample with that obtained from standard solution **(Figures 1 & 2)** and also by comparing retention factor (R_f =0.29). The peak corresponding to β-Sitosterol from the sample solution had the same retention factor as that of standard β-Sitosterol.

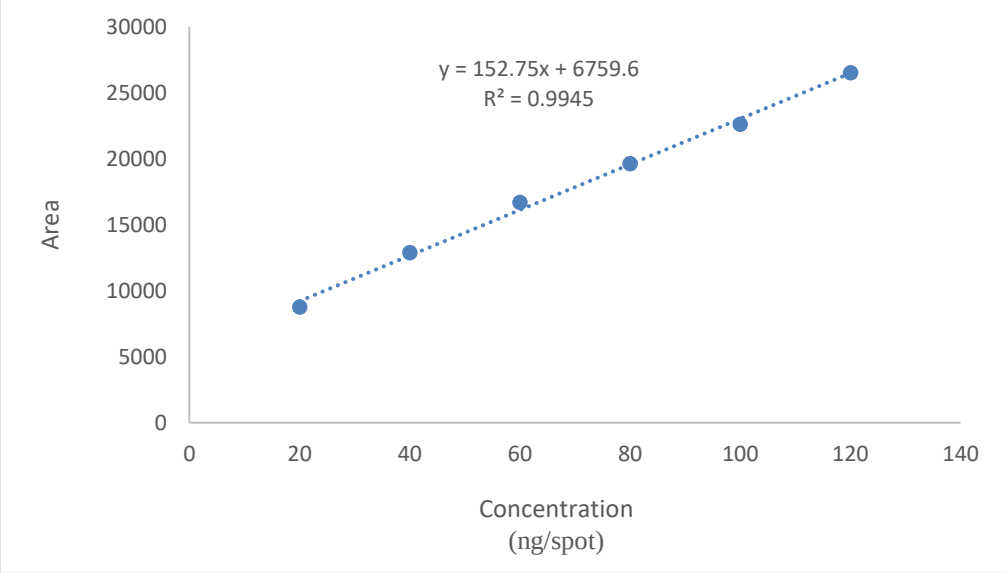
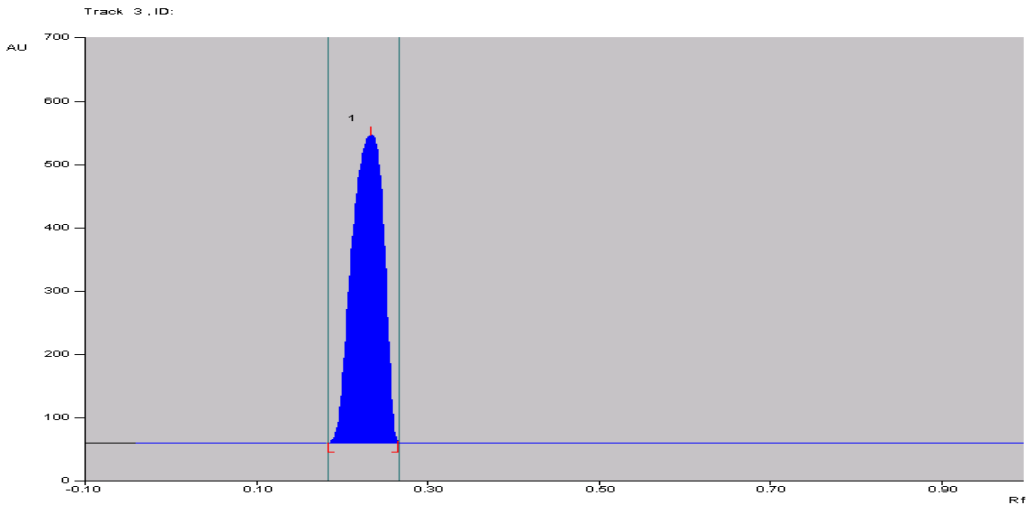
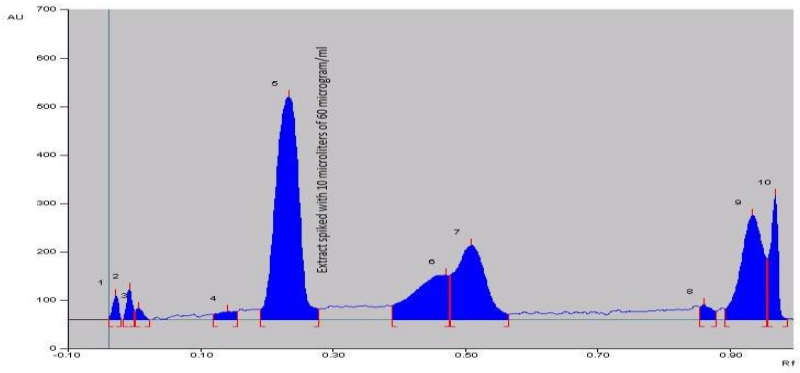


Figure 1. Calibration curve of Standard β- Sitosterol.



(a)



(b)

Figure 2. Typical HPTLC densitogram of β-sitosterol in Standard b) Solanum *xanthocarpum* fruit extract

3.1. Linearity

Calibration plot show in **(Figure 1)** indicates the response and the slope were 0.9945, 152.75and 6759.6 respectively **(Table 1).**

Table 1. Method validation data for HPTLC Estimation of β-Sitosterol.

Parameter	Value
Linearity range (ng/spot)	20–120
Correlation coefficient (r^2)	0.9945
Slope	152.75
Intercept	6759.6
LOD (ng/spot)	0.07
LOQ (ng/spot)	8.17
Repeatability (%RSD, n = 3)	0.77
Intermediate Precision (%RSD, n = 3)	0.33

3.2. LOD and LOQ

Signal to noise ratio 3 and 10 were considered for LOD and LOQ respectively and calculated from the slop of the calibration plot and the standard deviation of the response.LOD and LOQ were found to be 0.07 and 8.17ng/spot respectively for β-Sitosterol which ascertain the adequate sensitivity of the method **(Table 1).**

3.3. Precision

From the results of repeatability and intermediate precision studies experiments **(Table 1)** the developed method was found to be precise as % RSD values were found to be low (<2%).

3.4. Specificity

Specificity of the method was ascertained by comparing R_f values of the samples with that of standard β-Sitosterol.

3.5. Accuracy

The results of recovery studies of leaves extracts are listed in **(Table 2)**. The results are within acceptable limits demonstrating the accuracy of the method.

Table 2. Recovery studies of β-Sitosterol in leaf extract by HPTLC.

Level (%)	Recovery (%)	Overall Average Recovery (%)
80	97.63	
100	97.53	97.63
120	97.47	

3.6. Robustness

The low values of the % RSD (less than 2%) indicated after introduction of small changes in mobile phase composition, mobile phase volume and duration of mobile phase saturation time indicated the robustness of the method **(Table 3).**

Table 3. Robustness of the HPTLC method(n=3)

Parameter	%RSD
Mobile phase composition (Toluene: Ethyl acetate, 7:3)	1.08
Mobile phase volume (20 mL)	0.52
Duration of saturation (30 min)	0.74

4. CONCLUSION

A rapid, simple, accurate, specific, and robust HPTLC method for the quantitative estimation of β-Sitosterol 0.0872%w/w in the methanolic extract of leaves of *Solanum xanthocarpum* has been developed and validated. The developed method can be used as a tool for quantitative estimation of β-Sitosterol/herbal formulation.

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