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High-performance thin-layer chromatographic method for quantification of β-sitosterol from *Tridax procumbens*

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ABSTRACT

A simple, precise, accurate and reproducible high-performance thin layer chromatographic method has been established for quantification of β-sitosterol in whole plant powder of *Tridax Procumbens*. The amount of β-sitosterol in whole plant powder of *Tridax Procumbens* was found to be 0.04%. A methanolic extract of the whole plant powder was used for the experimental work. Sepration was performed on aluminium sheet silica gel 60 F_{254} HPTLC plates with Toluene: Ethyl acetate: Glacial acetic acid 8.0:2.0:0.2 (v/v), as solvent system. After development, plates were treated with Anisaldehyde Sulphuric Acid reagent. Detection and quantification were performed by densitometer at λ = 550nm. β-sitosterol response was linear over the range 10.0μg/ml to 70.0μg/ml. The validated HPTLC method can be used for a routine quality-control analysis of *Tridax Procumbens* whole plant powder and quantification of β-sitosterol.

KEYWORDS

HPTLC;
β-sitosterol;
Tridax Procumbens;
Anisaldehyde sulphuric acid reagent.

INTRODUCTION

Plant material or its extracts contains a complex mixture of different components and it is necessary to separate them before quantification. *Tridax Procumbens* commomly called as Mexican daisy is a semi-prostrate perennial herb belonging to family Asteraceae, growing wild in planes throughout India. It exhibits several pharmacological activities like used to stop bleeding, diarrheca, malaria, stomachache, pacifies vitiated pitta, inflammation, ulcers, anal fistula, and hemorrhoids, while its anti-diabetic, anti-hepatotoxic and anti-oxidant properties are recently revealed[1]. Reported chemical constituents in *Tridax Procumbens* are Sigmasterol and β-Sitosterol.

The literature reveals that there is no high-performance thin-layer chromatographic method available for quantification of β-sitosterol in whole plant powder of *Tridax Procumbens*. A simple, rapid, economical, precise, and accurate HPTLC method has been established for determination of β-sitosterol in *Tridax Procumbens* whole plant powder.

EXPERIMENTAL

Reagents and materials

Analytical grade Toluene, Ethyl acetate, Glacial Acetic Acid and Methanol were obtained from Qualigens Fine Chemicals, Mumbai, India. Standard β-sitosterol were procured from Sigma-Aldrich. The structure of

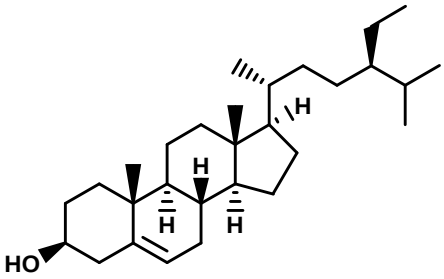

Figure 1 : Structure of β-sitosterol

β-sitosterol is shown in figure 1.

The plant *Tridax Procumbens* were collected from Bhandup (Mumbai), India and were authenticated by the National Botanical Research Institute (NBRI), Council of Scientific and Industrial Research, Lucknow, India.

Standard and sample preparation

Stock solution of β-sitosterol (1000μg mL^{-1}) was prepared by dissolving 10.310mg of 97.00% pure compound, transferring to a 10mL volumetric flask, dissolving in minimum quantity of methanol and diluting volume to the mark with the same solvent. Aliquots (0.1mL to 0.7mL) of this stock solution were transferred to 10mL volumetric flasks and the volume of each was made up to 10mL with methanol, to obtain working standard solutions containing 10μg mL^{-1} to 70μg mL^{-1}.

Tridax Procumbens plant were collected, washed, dried in the shade, powdered, and the powder was passed through an BSS 80-mesh sieve and stored in an airtight container at 25°C. 1.0g of dried plant powder was accurately weighed and placed in a stoppered tubes and 10mL of methanol was added, the samples were vortexed for 1-2 min and left to stand overnight at room temperature (28 ± 2°C). The contents of the tubes were filtered through Whatman No. 41 paper (E. Merck, Mumbai, India). The clear supernatants were collected in dry volumetric flask. This 0.1mg/ml solution of plant powder was used for the assay of β-sitosterol.

Instruments

A Camag Linomat IV sample applicator was used for sample application. Camag Twin trough glass chamber (20 × 10cm) was used for development of plates. Camag TLC scanner II equipped with cats 3 Version software was used for interpretation of data.

TABLE 1 : Linearity data for β-sitosterol

Data	β-sitosterol
Linearity range μg mL^{-1}	10 to 70
Slope (m)*	27.235
Intercept (c)*	-155.076
Correlation coefficient (R)	0.999
LOD μg mL^{-1}	5
LOQ μg mL^{-1}	10
Instrument Precision (RSD[%], n = 10)	1.06
Intraday Precision (RSD[%], n = 3)	0.16
Interday Precision (RSD[%], n =3)	0.14

***Of the equation y = mx + c, where y is peak area, m is the slope, x is the concentration and c is the intercept**

Chromatography

Procedure

Chromatography was performed on aluminium-backed HPTLC plates precoated with 0.2mm layers of silica gel 60 F_{254} (Merck # 5554); prewashing of plates was carried out using methanol and plates were dried in oven for 15 minutes. Samples (10μL) were applied on the plates as bands of 8mm width with the help of a Camag Linomat IV automatic sample applicator at the distance of 10mm from the bottom edge of the plates.

For β-sitosterol, plate was developed, at 25 ± 2°C, with Toluene: Ethyl acetate: Glacial acetic acid in the ratio 8.0:2.0:0.2 (v/v/v) as mobile phase in a Camag (Muttenz, Switzerland) glass twin- trough chamber, previously equilibrated with mobile phase for 15 minutes. The development distance was 8.0cm. After development, the plate was dried at room temperature and derivatized with Anisaldehyde sulphuric acid reagent in a derivatization chamber for 20 secs and again dried at room temperature. After drying, the plate was heated in oven at 105°C for 10 min before densitometric scanning.

The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape for β-sitosterol. The plates were scanned at λ = 550nm by means of Camag TLC Scanner and CATS3 software. A typical HPTLC chromatogram of β-sitosterol and plant is shown in figure 2. The chromatographic plate of β-sitosterol standard and *Tridax Procumbens* plant powder is shown in figure 3.

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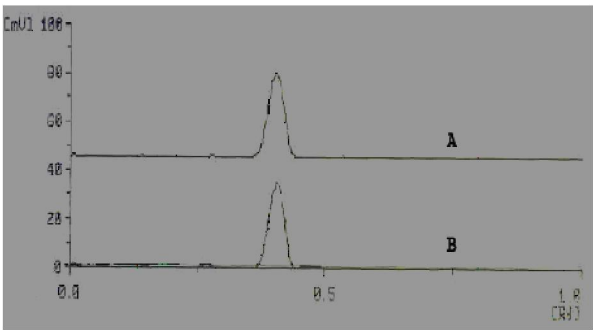


A Standard (β-sitosterol)
B Sample (*Tridax Procumbens* (Linn.))

Figure 2 : The overlain HPTLC chromatogram of β-sitosterol and *Tridax Procumbens* (Linn.)

Linearity of detector response

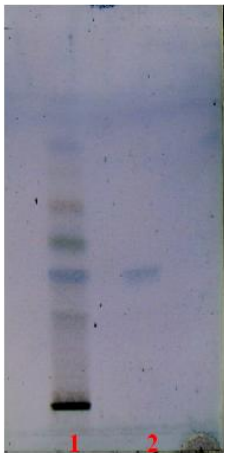
β-sitosterol solutions at seven different concentrations (10, 20, 30, 40, 50, 60 and 70μg mL^{-1}) were prepared in methanol. The above β-sitosterol solutions (10μL) were applied to HPTLC plates. The plates were developed as per the chromatographic condition mention in 2.4.1. The detector response for the different concentrations were measured. Graph were plotted of standard peak area against concentration of β-sitosterol. The plot was linear in the range 10 to 70μg mL^{-1} for β-sitosterol. The experiments were performed three times and the mean was used for the calculations. The linearity data is given in TABLE 1.

Assay

10μL of the standard solution of β-sitosterol (40μg mL^{-1}) and sample solutions were spotted on a HPTLC plate. The amount of β-sitosterol present in this solution was calculated by comparison of area measured for the sample to that for the standard. The assay procedures described earlier were repeated seven times starting from weighing of the whole plant powder. The amount of β-sitosterol found in plant powder of *Tridax Procumbens* was 0.04 % respectively.

Accuracy

The accuracy of the methods was established by performing recovery experiments by the standard addition method. The recovery of standard β-sitosterol added to *Tridax Procumbens* plant powder was studied at three different levels, each being analysed in a manner similar to that described for the assay. The contents of β-sitosterol were quantified by the proposed methods and the percentage recovery was calculated.



1) Sample (*Tridax Procumbens*)
2) Standard (β-sitosterol)

Figure 3 : Photograph of developed plate at 550nm

The recovery obtained for β-sitosterol standards was from 98.0% to 101.0%., showing the reproducibility of the methods was good. The average recovery for β-sitosterol was found to be 98.28 %.

RESULTS AND DISCUSSION

Of the different mobile phases investigated, Toluene: Ethyl acetate: Glacial acetic acid in the ratio 8.0:2.0:0.2 (v/v/v), resolved β-sitosterol (Rf = 0.40) very efficiently from the other components of the methanolic extract of *Tridax Procumbens*. The response of β-sitosterol was found to be linearly dependent on concentration in the range 10μg ml^{-1} to 70μg ml^{-1}, with correlation coeffient of 0.999.

The variability of the methods was studied by analyzing aliquots of the different concentrations of β-sitosterol solutions on the same day (intra-day precision) and on different days (inter-day precision) and by instrument precision. The results were expressed as % RSD. The % RSD values were found to be less than 2%, indicating that the selected method is precise and reproducible. The mean recovery of β-sitosterol was found to 98.28 % which indicates the accuracy of the methods.

The robustness of the methods was studied, during method development, by determining the effects of small variation, of mobile phase composition (±2%), duration of plate pre-washing, chamber saturation period, development distance and scanning time (10% variation of each). No significant change in R_F or in response

of β-sitosterol was observed, indicating the robustness of the methods.

CONCLUSION

The proposed method is simple, rapid, precise, accurate and economic and can be used for routine quality-control analysis of *Tridax Procumbens* plant powder and for quantitative determination of β-sitosterol from plant powder.

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