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Chromatographic techniques for quantification of sterol from *Mimosa pudica* Linn. whole plant powder

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ABSTRACT

A simple, precise, accurate and reproducible chromatographic methods has been established for quantification of β-sitosterol in whole plant powder of *Mimosa pudica* Linn. The amount of β-sitosterol in whole plant powder of *Mimosa pudica* Linn. was found to be 0.07mg. A methanol extract of the whole plant powder was used for the experimental work. Separation was performed using two different chromatographic techniques namely HPTLC and HPLC. β-sitosterol response was found to be linear over the range 1μg/mL to 20μg/mL in case of HPTLC and 0.5μg/mL to 20μg/mL in case of HPLC. Both the methods were validated and can be used for a routine quality–control analysis of *Mimosa pudica Linn.* whole plant powder and quantification of β-sitosterol.

KEYWORDS

HPTLC;
HPLC;
Quantification;
β-Sitosterol;
Mimosa pudica Linn.

INTRODUCTION

Mimosa pudica Linn. (Fam. -Leguminosae) the plant response to touch. This sensitive response of the leaves to touch is aptly called as touch me not plant. The plant grows widely as a rapidly growing shrub throughout India, in warm and humid regions. A diffuse prickly shrub, 30-120 cm in height value. The seeds, leaves, roots and the whole plant are used for medicinal purpose[1]. Internally, lajjalu is used in vast range of diseases. It is popular as indispensable drug for blood pressure[2] it also has larvicidal property[3]. It is used to treat menorrhagia and leucorrhoea[4,5]. Plant contains many chemical constituents like β-sitosterol (alkaloid), stigmasterol, leucoanthocyanidin, D-xylose and D-glucuronic acid, norepinephrine, D-pinitol, linoleic acid, oleic acid, palmitic acid, stearic acid, crocetin dimethyl ester[6].

β-sitosterol is a phytosterols or plant sterol. β-sitosterol is mainly known and used for its cholesterol lowering property[7]. It also has anticancer, antiulcer, antidiabetic, anti-inflammatory and antipyretic properties[8].

The literature reveals that there are no chromatographic methods available for quantification of β-sitosterol from whole plant powder of *Mimosa pudica Linn.* The aim of the work is to develop a simple, rapid, economical, precise, and accurate chromatographic methods for quantification of β-sitosterol from *Mimosa pudica Linn.* whole plant powder. The developed methods were further validated as per ICH guidelines to indicate its suitability[9,10].

EXPERIMENTAL

Materials

The plant *Mimosa pudica* L. was collected from

Mumbai, Maharashtra, India and was authenticated by National Institute for Science Communication and Information Resources (NISCAIR), New Delhi, India. HPLC grade methanol (99.0%) from S.D Fine Chemicals (India) was used. The double distilled water used was obtained by double distillation using Milli Q water purifying system (Millipore, USA). β-sitosterol standard was procured from Sigma-Aldrich Chemie Gmbh (Aldrich Division; Steinheim, Federal Republic of Germany).

Standard preparation

The stock solution (A) of β-sitosterol (1,000μg mL^{-1}) was prepared by dissolving 25mg of accurately weighed β-sitosterol in minimum quantity of methanol and diluting with same solution up to the mark in a 25mL standard volumetric flask. Further solution (B) of β-sitosterol (100μg mL^{-1}) was prepared by transferring 2.5mL of stock solution (A) and diluting with methanol in a 25mL volumetric flask. Different volumes in the range of 50-2000.0μL of stock solution (B) were transferred to 10mL standard volumetric flasks and diluted up to the mark with methanol, to provide a concentration range of 0.5-20.0μg mL^{-1}.

Sample preparation

The whole plant of *Mimosa pudica Linn.* was dried at room temperature and then ground in a mixer to a fine powder, which was passed through an ASTM BSS 85 mesh size. 1.0g of the sample powder was accurately weighed, placed in a 10mL volumetric flask, and was diluted with methanol up to the mark. After shaking, the flask was left overnight at room temperature. The content of the flask was then filtered through a Whatman No.41 paper and the clear filtrate was collected in another clean, dry, stoppered conical flask. This solution was used for the assay experiment.

Instrumentation and chromatographic conditions

HPTLC

High performance thin layer chromatography was performed on aluminium sheet precoated with silica gel 60 F_{254} HPTLC plates (Merck # 5554). Before use, plates were pre-washed with methanol and dried in an oven at 105°C for 1 hr. Samples (10μL) were applied on the plates as bands of 7mm width with the help of a Camag Linomat IV sample applicator at the distance of 15mm from the edge of the plates. The plates were developed to a distance of 80 mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 20 min. The solvent system was toluene: dichloromethane, (7.5: 2.5) (v/v). The chromatographic conditions as application mode, mobile phase, saturation time and band width had previously been optimized to achieve the best resolution and peak shape. After development, plates were dried under current of air at room temperature and derivatized with freshly prepared Anisaldehyde reagent in a derivatization chamber for 20 secs and dried at room temperature. After drying, plates were heated in oven at 105°C for 10 min before densitometric scanning[11]. Densitometric evaluation of the plates was performed at λ = 554nm using tungsten lamp with a Camag Scanner II in conjunction with Cats 3 software for quantification. The peak of β-sitosterol was obtained at Rf = 0.49. The overlay of the chromatograms of both standard and plant is given in figure 1 and figure 2.

HPLC

High performance liquid chromatographic was preformed with Merck Hitachi high performance liquid chromatograph equipped with L-7100 pump fitted with L-7455 auto Sampler and HSM-LACHROM Multi HSM manager chromatographic software was used for data acquisition. A waters symmetry shield C-18 column (150 × 4.6, 5μm) was used for the analysis. The mobile phase comprising of methanol: water in the ratio (95:5) v/v was filtered through a 0.45μm membrane filter (Millipore) and degassed by sonication. Throughout the run of 8.5 min. a flow rate of 1.0mL min^{-1} was maintained. The column effluent was monitored at 210nm with a L-2400 series multi-wavelength UV Detector. A typical HPLC chromatograms for determination of β-sitosterol form *Mimosa pudica* L. is shown in figure 3 and figure 4 respectively.

Method validation

System suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by using 10μL of standard solution of β-sitosterol (5μg mL^{-1}) six times. The % RSD was found to be 0.39 for

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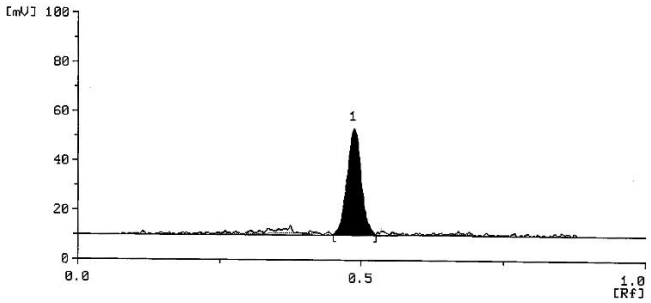

Figure 1 : HPTLC chromatogram for β-sitosterol standard

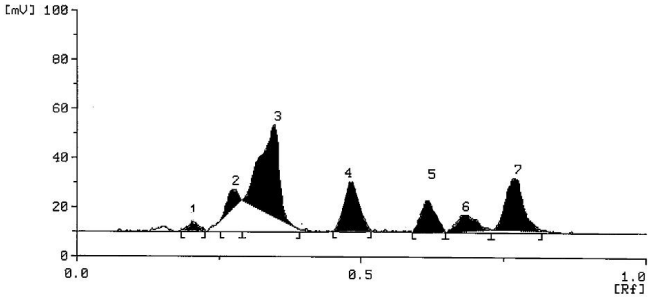

Figure 2 : HPTLC chromatogram for plant

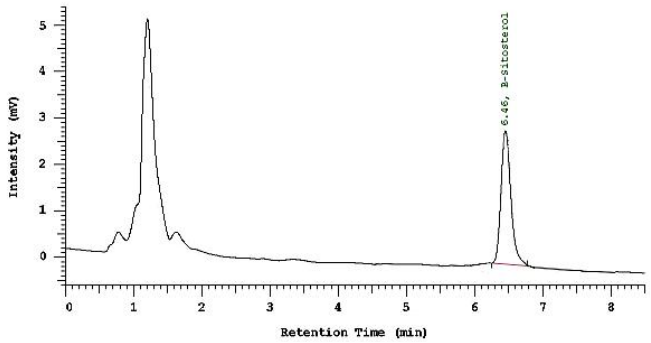

Figure 3 : HPLC chromatogram for β-sitosterol standard

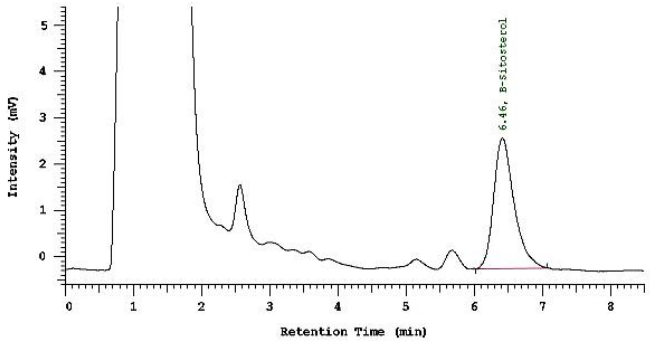

Figure 4 : HPLC chromatogram for plant

HPTLC and 0.42 for HPLC. The coefficient of variation (%RSD) was less than 2% for replicate measurement of the same sample. This shows that the method and the system are both suitable for quantification of β-sitosterol in unknown samples.

Limit of detection and limits of quantitation

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. The LOD and LOQ of β-sitosterol was 0.5μg mL^{-1} and 1.0μg mL^{-1} for HPTLC and 0.2μg mL^{-1} and 5.0μg mL^{-1} for HPLC respectively.

Linearity

In order to establish linearity using HPTLC technique, standard solutions of β-sitosterol at six different concentrations 1.0μg/mL to 20.0μg/mL were prepared in methanol. Each of these solutions (10μL) was applied to a plate, the plate was developed, the spots were derivatized, and the detector response for the different concentrations was measured. A graph was plotted of drug peak area against concentration of β-sitosterol. The plot was linear in the range 1.0μg/mL to 20.0μg/mL. In case of HPLC technique the standard solutions of β-sitosterol at six different concentrations 0.5μg/mL to 20.0μg/mL were prepared, 10μL of these solutions were injected and the plot was linear in this range. The experiment was performed three times for both the chromatographic techniques and the mean was used for the calculations. The data was analyzed by linear regression least squares fitting[12]. The statistical data obtained is given in TABLE 1.

TABLE 1 : Linearity

Parameters	HPTLC	HPLC
Linearity range	1.0 to 20.0μg/mL	0.5 to 20.0μg/mL
Slope (m)	158.5	21470.2
Intercept(c)	-13.4	-2890.7
Correlation coefficient R^2	0.9993	0.9997

Assay procedure

The developed HPTLC and HPLC methods were used for determination of β-sitosterol from whole plant powder of *Mimosa pudica* Linn. By following the developed chromatographic conditions as mentioned above the area of β-sitosterol peak in the sample working solution (10μL) was measured. From the calibration curve, the amount of β-sitosterol in dry powder of *Mimosa pudica* Linn. was calculated using both the techniques. In HPTLC R_f of β-sitosterol in sample solution was 0.48 and in the standard solution was found to be 0.49 and mean assay value was found to be 0.071mg/g with % RSD as 0.061%. Whereas in HPLC the retention time in sample solution was 6.46 and in

TABLE 2 : Results of recovery experiment

Technique	Level	Preanalysed sample in (μg mL^{-1})	Amount of std. added to preanalysed sample in (μg mL^{-1})	Total amount of std found in (μg mL^{-1})	SD	RSD (%) (n = 7)	Recovery (%)
	0	7.12	0	7.107	0.068	0.960	99.82
HPTLC	50 %	7.12	3.5	10.560	0.039	0.368	99.44
	100 %	7.12	5.0	13.919	0.027	0.197	98.58
						Mean	**99.28**
	0	6.972	0	6.938	0.071	1.023	99.50
HPLC	50 %	6.972	3.5	10.350	0.057	0.547	98.83
	100 %	6.972	5.0	13.856	0.038	0.277	99.17
						Mean	**99.17**

the standard solution was found to be 6.46 and the mean assay value was found to be 0.069mg/g of plant powder with % RSD as 1.101.

Precision and accuracy

The intra-day and inter-day precision was used to study the variability of the method. The % RSD for intra-day and inter-day precision for β-sitosterol were 0.48 and 0.67%, respectively for HPTLC and were 0.90 and 0.88% respectively for HPLC. Accuracy of the method was studied using the method of standard addition. Standard β-sitosterol was added to the extract of the whole plant powder and the percent recovery was determined at two different levels 50% and 100%. β-sitosterol content was determined and the percent recovery was calculated. The results of recovery analysis are shown in TABLE 2.

RESULTS AND DISCUSSION

The methods as described in the present work, results in two chromatographic techniques for quantification namely HPTLC and HPLC. HPTLC method utilizes pre-coated silica gel 60 F_{254} plates with toluene: dichloromethane, (7.5:2.5) (v/v), as mobile phase resulted in good separation of the drug from other phytochemicals. The peak of β-sitosterol was obtained at Rf = 0.49. Regression analysis of calibration data showed that the linearity of standard β-sitosterol was observed over a concentration range of 1.0μg/mL to 20.0μg/mL with regression coefficient of 0.9993. The amount of β-sitosterol in 10mL of sample solution was found to be 0.071mg. Whereas HPLC method as described in the present work, utilizes Merck Hitachi high performance liquid chromatograph equipped with L–7100 pump fitted with L-7455 auto Sampler and HSM-LACHROM Multi HSM manager chromatographic software was used for data acquisition. A waters symmetry shield C-18 column (150 × 4.6, 5μm) and mobile phase comprising of methanol: Water (95:5) *v/v* resulted in good separation. Regression analysis of calibration data for both showed that the linearity was observed over a concentration range of 0.5μg mL^{-1} to 20μg mL^{-1} with regression coefficient of 0.9997 and 0.9997 respectively. The concentration of β-sitosterol in 1.0g of whole plant powder of *Scoparia dulcis* L. was found to be 0.069mg.

Chromatographic methods are specific, sensitive, accurate, precise and reproducible. Thus these methods are preferred over other nonspecific techniques such as titrimetric and spectrophotometric methods. The proposed methods are simple and do not require elaborate sample preparation. Setting the optimized chromatographic parameters, HPTLC method is the simplest. The time required for equilibration of chromatographic conditions or changing a set of conditions is very short in HPTLC. HPTLC with photometric detection offers advantage that the solvents used in mobile phase do not affect quantification since they are removed by evaporation. Although this suggests that HPTLC is a better HPLC just cannot be neglected. From research point of view both methods seem to be effective for the actual purpose of developing and validating these methods. But the method involving HPLC will be preferred to HPTLC in various industries as the instrument mostly used in routine quality control is HPLC. Also a sincere effort has been made to reduce the cost factor involved while employing the HPLC method while trying not to compromise with the results that will be obtained.

Instrument precision, intra assay precision and intermediate precision were measured to evaluate the precision of both the methods. The low values of coefficient of variation are indicative of high precision of the method.

The accuracy of the methods was established by means of a recovery experiment. The mean recovery was close to 100 %, which indicates the accuracy of the methods. The low values of %COV for seven rep-

licate analyses are indicative of precision of the methods.

The proposed high performance thin layer chromatographic method and high performance liquid chromatographic method find applications in routine quality control analysis and also quantification of β-sitosterol.

CONCLUSION

The chromatographic methods developed for the quantification of β-sitosterol was found to be highly accurate and precise. It is suitable for the application of routine quality control analysis and quantification of β-sitosterol in *Mimosa pudica Linn.* whole plant powder. The linearity, precision, accuracy of the methods proves that the method are easily reproducible in any quality control set-up provided all the parameters are followed accurately.

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