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Simultaneous determination of β-sitosterol and lupeol from *Scoparia dulcis* L. by high-performance liquid chromatography

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ABSTRACT

A simple, precise and accurate high-performance liquid chromatographic method has been developed for simultaneous determination of β-sitosterol and lupeol in whole plant powder of *Scoparia dulcis* L. Chromatographic separation of the two drugs was performed on a thermo hipurity advance column (150×4.6, 5μm) as stationary phase with a mobile phase comprising of methanol : 0.1% O-phosphoric acid (90:10) *v/v* at a flow rate of 1.0mL min^{-1} and UV detection at 210nm with a run time of 20.0 min. The proposed method was validated for linearity, accuracy, precision and limit of quantitation. The validated HPLC method can be used for a routine quality control analysis and simultaneous quantitation of β-sitosterol and lupeol from *Scoparia dulcis* L.

KEYWORDS

HPLC;
β-sitosterol;
Lupeol;
Scoparia dulcis L.

INTRODUCTION

Scoparia dulcis L. (Family: *Scrophulariaceae*) commonly known as Sweet Broomweed is widely used in Indian folk medicine for the treatment of diabetes mellitus[1]. This plant has been also used to cure many liver aliments. Phytochemical screening has revealed that the plant contains diterpenoids, flavonoids, tannins, alkaloids, triterpenes, hexacosonol, β-sitosterol, ketone-dulcitone, and amellin, an antidiabetic compound[2].

β-sitosterol is a phytosterols or plant sterol. β-sitosterol is mainly known and used for its cholesterol lowering property. But studies have shown that the phytochemical may have other health benefits: easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity[3]. Lupeol, a triterpene has been reported to possess a wide range of medicinal properties that include strong antioxidant, antimutagenic, anti-inflammatory and antiarthritic effects[4].

Literature reveals that there are different methods available for determination of β-sitosterol and lupeol from different sources[5-8], but there is no such high-performance liquid chromatographic method available for simultaneous determination of β-sitosterol and lupeol from *Scoparia dulcis* L. whole plant powder. Plant material or its extracts contains a complex mixture of different components and it is necessary to separate them before quantification. The aim of the work was to develop a simple, sensitive, economical, precise, and

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accurate HPLC method for simultaneous determination of β-sitosterol and lupeol from *Scoparia dulcis* L. The method was further validated as per ICH guidelines[9,10].

EXPERIMENTAL

Chemicals and preparation of standard solutions

HPLC grade methanol (99.0%), orthophosphoric acid (85%) from S.D Fine Chemicals (India) were used. The double distilled water used was obtained by double distillation using Milli Q water purifying system (Millipore, USA). β-sitosterol and Lupeol standards were procured from Sigma-Aldrich Chemie Gmbh (Aldrich Division; Steinheim, Federal Republic of Germany). Stock solutions of β-sitosterol and Lupeol (1000μg mL^{-1}) were prepared separately by accurately weighing 10 mg of both standards, transferring to two different 10mL volumetric flask, dissolving in minimum quantity of methanol and diluting to volume with the same solvent. Further the solutions containing the mixture of β-sitosterol and Lupeol were prepared using these stock solutions. The concentration ranges for both, β-sitosterol and Lupeol in working standard solutions were 1.0μg mL^{-1} to 20μg mL^{-1}.

Plant material and preparation of sample solution

The plant *Scoparia dulcis* L. was collected from Tarapur, Maharashtra, India and was authenticated by National Botanical Research Institute (NBRI), Council of Scientific and Industrial Research, Lucknow, India. The collected plant material was dried at room temperature in shade and then ground in a mixer to a fine powder, which was passed through an ASTM BSS 85 mesh size and stored in an airtight container, at room temperature. 500mg of the accurately weighed, dried whole plant powder was transferred to a 10mL standard volumetric flask and made up to the mark with methanol. The solution was vortexed for 5 min and then left to stand overnight at room temperature. The solution was filtered through Whatman filter paper no 41 (E. Merck, Mumbai, India). The filtrate was collected in a dry stoppered test tube and used.

Instrumentation and chromatographic conditions

Chromatographic separation 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 thermo hipurity advance column (150×4.6, 5μm) was used for the analysis. The mobile phase comprising of methanol: 0.1% O-phosphoric acid in the ratio (90:10) v/v was filtered through a 0.45 μm membrane filter (Millipore) and degassed by sonication. Throughout the run a flow rate of 1.0mL min^{-1} was maintained. The column effluent was monitored at 210 nm with a L-2400 series multi-wavelength UV Detector. A typical HPLC chromatograms for simultaneous determination of β-sitosterol and Lupeol form *Scoparia dulcis* L. are shown in figure 1 and 2 respectively.

Method validation

System suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by injecting 10μL of mixture of standard solution of β-sitosterol and Lupeol (5μg mL^{-1}) six times. The % RSD was found to be 0.17 for β-sitosterol and 0.40 for Lupeol, which was acceptable as it is less than 2%.

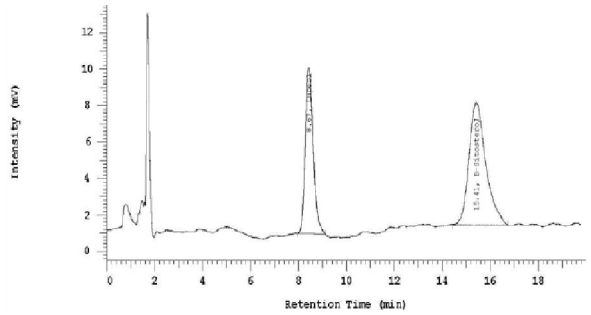

Figure 1 : HPLC chromatogram for β-sitosterol and lupeol standard

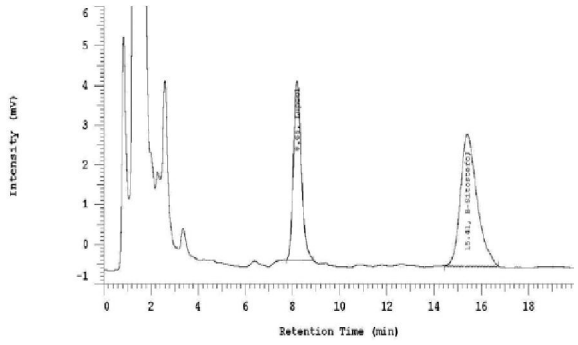

Figure 2 : HPLC chromatogram for plant *Scoparia dulcis* L.

Linearity

In order to establish linearity, mixture of standard solutions of β-sitosterol and Lupeol at six different concentrations (1.0, 3.0, 5.0, 10.0, 15.0 and 20μg mL^{-1}) were prepared in methanol. Each of these solutions (10μL) was injected and the detector response for the different concentrations was measured for both the drugs. A graph was plotted of drug peak area against concentration for both the drugs. The plot was linear in the range 1.0μg mL^{-1} to 20μg mL^{-1} for both the drugs. The experiment was performed three times and the mean was used for the calculations. The data was analyzed by linear regression least squares fitting. The statistical data obtained is given in TABLE 1.

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} and Lupeol was 0.5μg mL^{-1} and 1.0μg mL^{-1} respectively.

Assay

The developed HPLC method was used for simultaneous determination of β-sitosterol and lupeol from whole plant powder of *Scoparia dulcis* L. The sample working solution (10μL) was injected and the area of both β-sitosterol and Lupeol peak was measured. From the calibration curve, the amount of β-sitosterol and lupeol in dry powder of *Scoparia dulcis* L. was calculated. The retention time of β-sitosterol and Lupeol in sample solution was 15.41 and 8.65 and in the standard solution was found to be 15.41 and 8.67 respectively. The mean assay value of β-sitosterol was found to be 0.071mg per 500mg of plant powder with % RSD as 0.037 and mean assay value of lupeol was found to be 0.039mg per 500mg of plant powder with % RSD as 0.771.

TABLE 1 : Results of linearity

Standard	Slope (mean)	Intercept (mean)	Correlation coefficient
β-sitosterol	15915.7	3005.2	0.9992
Lupeol	12503.2	-579.7	0.9997

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 0.38 and 0.23%, respectively and Lupeol were 0.36 and 0.25%, respectively. Accuracy of the method was studied using the method of standard addition. Standard β-sitosterol and Lupeol solutions were added to the extract of the whole plant powder of *Scoparia dulcis* L. and the percent recovery was determined at two different levels 50% and 100%. β-sitosterol and Lupeol content was determined and the percent recovery was calculated. The results of recovery analysis are shown in TABLE 2 for both β-sitosterol and Lupeol.

RESULTS AND DISCUSSION

The 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 thermo hipurity advance column (150×4.6, 5μm) and mobile phase comprising of methanol: 0.1%

TABLE 2 : Results of recovery experiment

Standard	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 (%)
β-sitosterol	0	7.059	0	7.055	0.010	0.148	99.95
	50%	7.059	3.5	10.565	0.006	0.058	100.06
	100 %	7.059	7.0	13.929	0.029	0.207	99.08
						Mean	99.69
Lupeol	0	3.932	0	3.920	0.040	1.020	99.70
	50%	3.932	2	5.907	0.023	0.391	99.58
	100%	3.932	4	7.898	0.013	0.165	99.57
						Mean	99.61

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O-phosphoric acid in the ratio (90:10) *v/v* resulted in good separation of both β-sitosterol and lupeol from other phytochemicals. Regression analysis of calibration data for both showed that the linearity of β-sitosterol and lupeol was observed over a concentration range of 1.0μg mL^{-1} to 20μg mL^{-1} with regression coefficient of 0.9992 and 0.9997 respectively. The concentration of β-sitosterol in 1.0g of whole plant powder of *Scoparia dulcis* L. was found to be 0.141mg and that of lupeol was found to be 0.079mg.

When the method was validated in terms of instrumental precision, intra-assay precision and intermediate precision, the percent RSD values were found to be less then 2, indicating that the proposed method is precise and reproducible. The accuracy of the method was established by means of recovery experiments. The mean recovery was close to 100%, which indicates that method is accurate for simultaneous determination of β-sitosterol and lupeol. The low values of %COV for seven replicate analyses are indicative of precision of the method. The method is specific because it resolved the standard β-sitosterol and lupeol (Retention time = 15.41 and 8.65) well in presence of other phytochemicals of whole plant powder of *Scoparia dulcis* L.

CONCLUSION

A new HPLC method has been developed for the simultaneous estimation of β-sitosterol and lupeol from the methanolic extract of whole plant powder of *Scoparia dulcis* L. The HPLC method developed with careful validation was found to be simple, precise, economic, sensitive and accurate. The linearity, precision, accuracy of the method proves that the method is easily reproducible in any quality control set-up provided all the parameters are followed accurately.

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