ISSN 0975-6299 **Vol.1/Issue-3/Jul-Sep.2010**

International Journal of Pharma and Bio Sciences

DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF B-SITOSTEROL AND LUPEOL IN *VERNONIA CINEREA* LINN.

WILLY SHAH*[1], M.B.KEKARE[2] AND VIKAS VAIDYA[1]

[1]Department of Chemistry, Ramnarain Ruia College, Matunga, Mumbai-400 019.
[2]Department of Chemistry, Kirti College, Dadar, Mumbai-400 028.

**** Corresponding Author*** willy_shah@yahoo.com

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 *Vernonia cinerea* Linn. Chromatographic separation of the two compounds was performed on a waters symmetry shield C18 column (150 x 4.6, 5 µm) as stationary phase with a mobile phase comprising of methanol : acetonitrile (30:70) *v/v* at a flow rate of 1.0 mL min^{-1} and UV detection at 210nm with a run time of 12.0 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 *Vernonia cinerea* Linn.

KEY WORDS

HPLC, β-sitosterol, Lupeol, *Vernonia cinerea* Linn.

INTRODUCTION

Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the implements in the acceptance of the Ayurveda or Siddha formulations is the lack of standard quality control profile[1]. The quality of herbal medicine that is the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and modern analytical techniques are accepted to help in circumventing this problem[2]. Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization. HPLC has preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability for thorough screening etc[3].

Vernonia cinerea Linn. (Fam. Asteraceae); an erect, rarely decumbent, branched herb, 12-75 cm high, found throughout India ascending to an altitude of 1800 m. (known as Sahadevi in Sanskrit). It is considered to posses anti-inflammatory, antihelmintic, diuretic

activity, and it is used against skin diseases[4]. HPLC methods, hitherto, has not been reported for simultaneous estimation of β- sitosterol and Lupeol from *Vernonia cinerea* Linn. In this paper development and validation of a HPLC method for the quantitative analysis β- sitosterol and Lupeol is reported. The proposed method has been validated as per ICH guidelines[5-7].

EXPERIMENTAL

Plant material and Sample Preparation

Whole plant of *Vernonia cinerea* Linn. was collected from Matunga (Mumbai) region of India during the flowering season. It was authenticated from Blatter Herbarium, St. Xavier's College, Mumbai, India. After collection, 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. 250 mg of whole plant material of *Vernonia cinerea* Linn. was extracted with 10 ml of methanol. The mixture was vortexed for 5 min and it was overnight for extraction. methanol. It was filtered through Whatmann filter paper No. 1 and it was subjected to HPLC for simultaneous quantitation of β-sitosterol and Lupeol.

Chemicals and standard solutions Preparation

All the chemicals used in the experiments were of HPLC grade. Reference standard β- sitosterol and Lupeol (purity 98%) were procured from Sigma Aldrich (Germany). The stock solutions of β- sitosterol and Lupeol (10mg ml^{-1}) each were prepared separately in methanol. The stock solution were quantitatively transferred to give a solution of appropriate concentration range of β- sitosterol (7 µg mL^{-1} – 13 µg mL^{-1}) and lupeol (21 µg mL^{-1} – 39 µg mL^{-1}) respectively. Standard solutions were prepared by dilution of the stock solution.

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 waters symmetry shield C18 column (150 x 4.6, 5 µm) was used for the analysis. The mobile phase comprising of methanol: acetonitrile in the ratio (30:70) v/v was filtered through a 0.45 µm membrane filter (Millipore) and degassed by sonication. Throughout the run a flow rate of 1.0 mL min^{-1} was maintained. The column effluent was monitored at 210 nm with L-2400 series multi-wavelength UV Detector. A typical HPLC chromatograms of whole plant powder of *Vernonia cinerea* Linn. And for simultaneous determination of β-sitosterol and Lupeol standards are shown in Figure 1 and Figure 2 respectively.

METHOD VALIDATION

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by injecting 10 µL of mixture of standard solution of assay concentration of β-sitosterol and Lupeol six times. The % RSD was found to be 0.75 for β-sitosterol and 0.32 for Lupeol, which was acceptable as it is less than 2%.

Linearity

A good linearity was achieved in the concentration ranges of 7 µg mL^{-1} – 13 µg mL^{-1} for β-sitosterol and 21 µg mL^{-1} –39µg mL^{-1} for Lupeol. The regression equations and correlation coefficient for the reference were $y = 626.1x + 327.81$, $R^2 = 0.9986$ for β-sitosterol and $y = 969.8x - 1512.1$, $R^2 = 0.9992$ for Lupeol respectively. 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.

Table 1.
Method validation summary

Parameters	β-sitosterol	Lupeol
Linearity range [µg mL^{-1}]	7 µg mL^{-1} – 13 µg mL^{-1}	21 µg mL^{-1} – 39 µg mL^{-1}
Slope (*m*)[a)]	626.1	969.8
Intercept(*c*) [a)]	327.81	-1512.1
Correlation coefficient (R)	0.9986	0.9992
LOD [µg mL^{-1}]	0.5	7
LOQ [µg mL^{-1}]	1.5	10
Intraday precision (n=3 COV)	0.85%	0.35%
Interday precision (n=3 COV)	0.80%	0.63%
System Suitability	0.75%	0.32%

[a)] of the equation $y = mx + c$, where y is peak area, m is the slope, x is the concentration, and c is the intercept.

Table 2.

Recovery study Results

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	12.29	0	12.23	0.14	1.10	99.51
	50%	12.29	6.14	18.4	0.10	0.56	99.78
	100%	12.29	12.29	24.47	0.10	0.42	99.55
						Mean	99.69
Lupeol	0	37.58	0	37.6	0.15	0.39	100.05
	50%	37.58	18.79	56.26	0.17	0.30	99.80
	100%	37.58	37.58	75.03	0.16	0.22	99.83
						Mean	99.90

*** Mean ± SD, n= 7**

Table 3.
Assay Results

Sample Tested	Content of Marker compound* in mg	
	β-sitosterol	Lupeol
Whole plant of powder of *Vernonia cinerea* Linn with β- sitosterol and Lupeol.	0.1229	0.3758

*** Mean ± SD, n= 3**

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 0.5 µg mL^{-1} and 1.5µg mL^{-1} and Lupeol was 7.0 µg mL^{-1} and 10.0 µg mL^{-1} respectively.

Assay

The developed HPLC method was used for simultaneous determination of β-sitosterol and lupeol from whole plant powder of *Vernonia cinerea* Linn. The sample working solution (10 µL) 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 *Vernonia cinerea* Linn. was calculated. The retention time of β-sitosterol and Lupeol in sample solution was 7.06 and 4.45 and in the standard solution was found to be 7.08 and 4.48 respectively. The mean assay value of β-sitosterol was found to be 0.1229 mg per 250 mg of plant powder with % RSD as 1.2540 and mean assay value of lupeol was found to be 0.3758 mg per 250 mg of plant powder with % RSD as 0.8153.

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 0.85 and 0.80%, respectively and Lupeol were 0.35 and 0.63 % 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 *Vernonia cinerea* Linn. 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.

Figure 1.

Chromatogram of whole plant powder of Vernonia cinerea Linn.

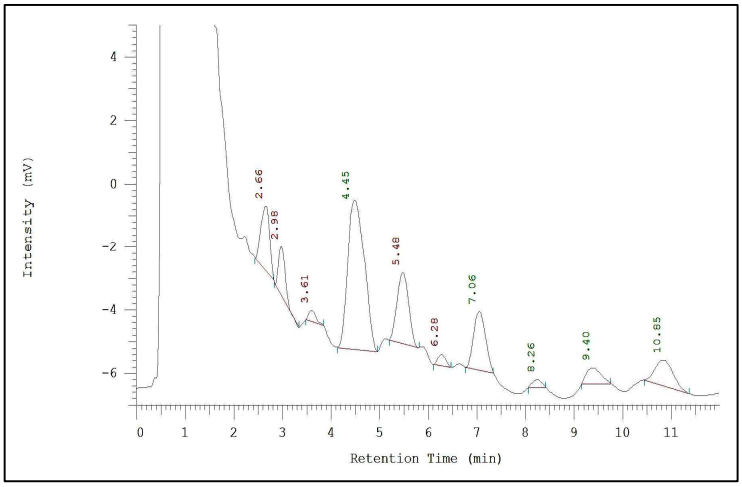
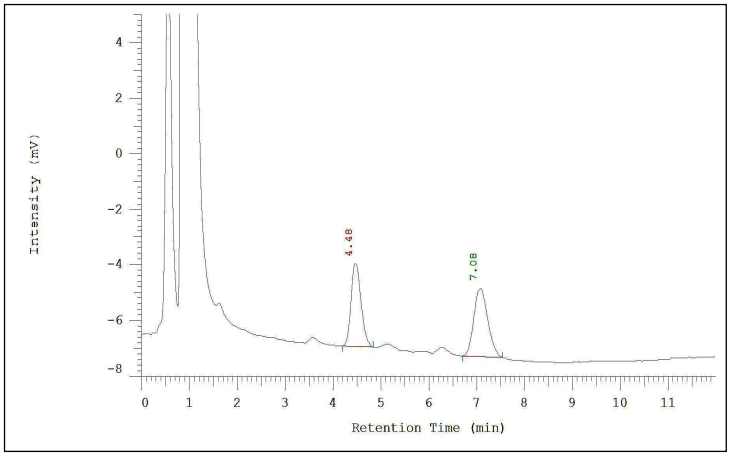


Figure 2.

Chromatogram of Standards



CONCLUSION

The application of a simple, rapid and accurate HPLC method for the quantitation of β- sitosterol and Lupeol in whole plant powder of *Vernonia cinerea* Linn. The method was validated to track the active principles in the complex mixture of herbal ingredients. The method could be extended for the marker-based standardization of other herbal product containing β- sitosterol and Lupeol. The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and for the quantification of these compounds in plant materials.

REFERENCES

1. M.S. Bagul, and M. Rajani, 2005, Phytochemical evaluation of classical formulation. A case study, Indian Drugs, Vol. 42, page- 15- 19.
2. Natalie J. Lazerowych and Peter P. use of fingerprinting and marker compounds for identification and standardization of botanical drugs. Strategies for applying pharmaceutical HPLC analysis to herbal products. Drug information journal, 32; 497-512, 1998.
3. Practical HPLC method development Lloyed Snyder, Joseph Kirkland, Joseph glajch, A wiley interscience publication, 2nd edition.1997.
4. Ayurvedic Pharmacopoeia of India, Part-1, Vol.3, 2nd edition. New Delhi: Government of India, Ministry of Health and Family Welfare, Department of Health; 2001; p.142-144. ISBN: 81-901151-5-4.
5. ICH, Q2A, 1994, Validation of Analytical Procedure: Methodology, In. Proc. Int. Con. Harmonization, Geneva.
6. ICH Q2B, 1996, Validation of Analytical Procedure: Methodology, In. Proc. Int. Con. Harmonization, Geneva.
7. Validation of analytical procedures: text and methodology Q2(R1), ICH Harmonised Tripartite Guideline, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, November 2005.