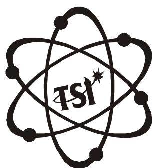

Trade Science Inc.

August 2008 Volume 7 Issue 8

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 7(8) 2008 [606-608]

High performance thin layer chromatographic method for the quantification of lupeol in bark powder of *Bauhinia variegata* linn

M.B.Kekare[1]*, N.Bhandarkar[1], V.V.Vaidya[2], G.Charegaonkar[2]

[1]Department of Chemistry, Kirti M.Dongursee College, Dadar, Mumbai-400028, (INDIA)

[2]Department of Chemistry, S.P.Mandali's Ramnarain Ruia College, Matunga, Mumbai-400019, (INDIA)

Tel : 09322404966

E-mail : mpc26@rediffmail.com

Received: 17th July, 2008 ; Accepted: 22nd July, 2008

ABSTRACT

A sensitive, rapid, simple, and accurate high performance thin layer chromatographic method has been developed to standardize the bark of *Bauhinia variegata* Linn.(Family: Caesalpiniaceae) using lupeol as an analytical marker. The ingredients from the bark powder were extracted with methanol which was used for quantification. Chromatography was performed on HPTLC silica gel $60F_{254}$ plates with toluene-acetone-acetonitrile, 9+0.8+0.2 (v/v), as mobile phase. Under these conditions, the R_f of lupeol was 0.58. Quantification was achieved by densitometric scanning at λ_{max}= 520nm in normal mode. The response to lupeol was a linear function of concentration over the range 5 to 35μg mL^{-1}. The amount of lupeol in bark powder of *B.variegata* Linn. was found to be 0.10 mg g^{-1}. The method permits reliable quantification of lupeol and good resolution and separation of lupeol from other constituents of *B.variegata* Linn. The method was validated for system suitability, linearity, precision and accuracy.

© 2008 Trade Science Inc. - INDIA

KEYWORDS

HPTLC;
Lupeol;
Quantification;
Bauhinia variegata Linn;
Bark powder.

INTRODUCTION

B.variegata Linn. (Caesalpiniaceae) is a medium sized deciduous tree found in the Sub-Himalayan tract and Outer Himalayas of the Punjab, Kumaon, Sikkim ,India, Burma, China[1]. The bark is used for the treatment of leucoderma, leprosy, asthma. The bark of *B.variegata* Linn. contains hentriacontane, octacosanol, signasterol, β-sitosterol, lupeol and a flavanoneglycoside -5,7-dimethoxyflavanone, 4′-o-α-L-rhamnopyranosyl-β-D-glucopyranoside[2].5,7-Dihydroxyflavanone-4′-O-α-L-rhamnopyranosyl-β-D-glucopyranoside, a flavanone glycoside from ethanolic extract of the stem of *B.variegata* Linn. has been isolated[3]. Phytochemical examination of the stem of *B.variegata* Linn. resulted in the isolation and identification of β-sitosterol, lupeol and an unknown compound naringenin 5,7-dimethyl ether 4′-rhamnoglucoside[4]. A flavanone glycoside from ethanolic extract of the stem of *B.variegata* has been characterized as kaempferol-3-glucoside on the basis of spectral data. The sugars and amino acid present in the stem have also been isolated and identified[5]. Stem bark of *B.variegata* yielded four substances- hentriacontane, octacosanol, β-sitosterol and stigmasterol[6]. A lot of research has been carried out individually using lupeol as a marker and *B.variegata* Linn. as the plant. But an extensive literature survey reveals no availability of HPTLC method for quantification of lupeol in bark powder of *B.variegata* Linn. Lupeol is freely soluble in ether, benzene, petroleum ether, warm alcohols but practically insoluble in water, dilute acids and alcohols[7]. Hence, a simple and reliable HPTLC

method has been established for the same.

EXPERIMENTAL

Materials

B.variegata Linn. was collected from (Mumbai, India) and authenticated. Standard lupeol (95% purity) was procured from Sigma-Aldrich Chemie (Steinheim, Germany). Toluene, acetone and acetonitrile were of analytical grade and were purchased from (Merck, India). Distilled water was used for analysis.

Standard and sample preparation

A stock solution of lupeol (1000 μg mL^{-1}) was prepared by dissolving 25.0 mg accurately weighed lupeol and diluting to 25.0 mL with methanol. Aliquots (0.05 to 0.35 mL) of the stock solution were transferred to 10 mL standard volumetric flasks and diluted to volume with methanol to obtain the working standard solutions containing 5 to 35 g mL^{-1}. The bark powder of *B.variegata* Linn. was collected, washed, dried in the shade, powdered, sieved through an 80-mesh sieve, and stored in an air tight container at room temperature. The dried powder (1000mg) was accurately weighed, placed in a volumetric flask and 10 mL methanol was added. The contents were first vortexed for 1-2 mins and then left to stand overnight at room temperature. The contents were then filtered through Whatman no. 41 paper (Merck, India). The filtrate was used for quantification and validation.

Chromatography

Procedure

Chromatography was performed on HPTLC silica gel $60F_{254}$ plates from (Merck, India).Samples (10μL) were applied to the plates as bands 8mm wide, 15mm from the edges, by means of a Camag (Muttenz, Switzerland) Linomat 5 sample applicator. The plates were developed to a distance of 70mm with toluene-acetone-acetonitrile, 9+0.8+0.2 (v/v), as mobile phase in a Camag twin trough chamber. The developed plates were dried with the help of a dryer. The developed plates were then derivatized with anisaldehyde-sulphuric acid reagent by dipping the plates in the dipping chamber. The plates were then heated on a Camag TLC plate heater 3 at 110^{0}C for 10 mins. Densitometric evaluation of the plates was performed at λ =520nm using tungsten lamp with the help of Camag TLC scanner 3 in conjunction with winCATS software, version 1.4.3[8].The wavelength used for densitometry was selected after acquiring spectra of the standard and the sample. Typical chromatograms obtained from the standard and the sample are shown in figure 1. A chromatographic plate obtained from lupeol standard and *B.variegata* Linn. is shown in figure 2.

Linearity of detector response

Solutions containing lupeol at seven different concentrations (5,10,15,20,25,30,35μg mL^{-1}) were prepared in methanol. Each of these solutions (10μL) was applied to a plate, the plate was developed, derivatized and the detector response to the different concentrations was measured. A graph of peak area against concentration of lupeol was plotted. The plot was linear in the range 5 to 35μg mL^{-1}. The experiment was performed three times and the mean was used for the calculations. The linearity data is given in TABLE 1.

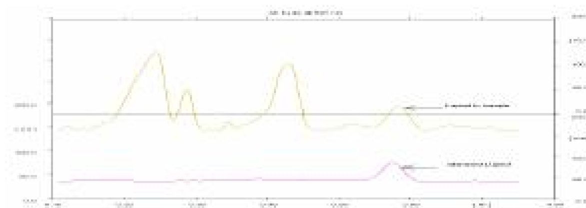

Figure 1: Typical HPTLC chromatograms obtained from lupeol standard and extract of *B.variegata* Linn

Figure 2: HPTLC plate after chromatography of a lupeol standard (T1) and extract of *B.variegata* Linn (T2)

TABLE 1 : Result of linearity

Linearity range (μg Ml^{-1})	5 to 35
Slope (m)a)	88.38
Intercept(c)a)	14.66
Correlation coefficient(R)	0.9999
LOD [μg mL^{-1}]	0.5
LOQ [μg mL^{-1}]	5
Instrumental Precision (RSD [%], n=5)	0.11
Intra-day Precision (RSD [%], n=3)	0.11
Inter-day Precision (RSD [%], n=3)	0.09

Full Paper

TABLE 2 : Results from assay of bark powder of *B.variegata* Linn

Weight of sample(mg)	1000
Amount of lupeol in sample(mg)	0.10
Average lupeol content (%)	0.010

TABLE 3: Results from determination of accuracy

Amount of lupeol in preanalyzed sample (mg)	0.10	0.10	0.10
Amount of lupeol added to preanalyzed sample (mg)	0	0.05	0.10
Total amount of lupeol (mg)	0.100	0.151	0.199
Recovery (%)	100.9	100.9	99.7
Mean recovery (%)		100.5	

Assay procedure

Lupeol standard solution and 10µL extract of bark powder of *B.variegata* Linn. were spotted on a plate. The amount of lupeol in the standard solution was calculated by comparing the areas of the standard and the sample. The assay procedure described above was repeated seven times. The mean amount of lupeol in 1000 mg bark powder was 0.10 mg g^{-1}. The results obtained from the assay are given in TABLE 2.

Accuracy

The accuracy of the method was established by performing recovery experiments using the method of standard additions. Lupeol was added at two different concentrations to the extract of bark powder of *B.variegata* Linn. and each was analyzed as described above. The lupeol content and the percentage recovery were calculated. The results are given in TABLE 3.

RESULTS AND DISCUSSION

Lupeol was accurately quantified in the sample using HPTLC silica gel plates $60F_{254}$, with toluene-acetone-acetontrile,9+0.8+0.2 (v/v),as mobile phase. The identity of lupeol was confirmed by overlaying the chromatograms of the plant extract with that of the standard (R_f-0.58). The detector response was a linear function of concentration over the range 5 to 35 µg mL^{-1}. The amount of lupeol in bark powder of *B.variegata* Linn. was found to be 0.10 mg g^{-1}.

System suitability was studied. The RSD values were less than 2% indicating that the complete testing system was suitable for the quantification and validation of lupeol in the bark powder of *B.variegata* Linn. Instrumental precision, inter-day precision and intra-day precision were measured. RSD values were less than 2%, indicating the method is precise and reproducible.Percentage recovery was 100.5%. Percentage recovery from 98 to 101 show the excellent reliability and reproducibility of the method.

CONCLUSIONS

The HPTLC method for the quantification and validation of lupeol from the bark powder of *B.variegata* Linn. is simple and reliable. The proposed HPTLC method for quantitative monitoring of lupeol in *B.variegata* Linn.can be used for routine quality testing.

ACKNOWLEDGMENTS

The authors wish to thank the staff of Anchrom Pvt Ltd, Mulund (Mumbai), India for their assistance with the project.

REFERENCES

[1] K.R.Kirtikar, B.D.Basu; Indian Medicinal Plants, **2**, 898-900.
[2] Husaina; Dictionary of Indian Medicinal Plants, **70**, **(1992)**.
[3] A.K.Gupta, T.J.Vidyapatil, J.S.Chauhan; Indian J.Chem., **18B**, 85-86 **(1979)**.
[4] A.K.Gupta, T.J.Vidyapatil, J.S.Chauhan; Planta Medica, **38**, 174-176 **(1980)**.
[5] A.K.Gupta, J.S.Chauhan; Natl.Acad.Sci.Lett., **7(1)**, 15-16 **(1984)**.
[6] Anand Prakash, R.L.Khosa; J.Res.Indian Med. Yoga Homoeop., **13(3)**, 96-97 **(1978)**.
[7] The Merck Index, **14th** Ed., **(1972)**.
[8] Quality Standards of Indian Medicinal Plants, **4**, **268** **(1978)**.