

Development of HPTLC Method for Puerarin Estimation in *Pueraria tuberosa* (Roxb.ex Willd.) DC.

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Abstract: A simple, precise, and rapid HPTLC method has been established for analysis of puerarin from ethanolic extract of tubers of *Pueraria tuberosa*. The method involves densitometric evaluation of puerarin after their resolution on pre-coated silica gel 60F254 plates with chloroform-methanol 9:1 (v/v) as mobile phase. Detection was at 254 nm. Good resolution was achieved with R_f 0.71 for puerarin. The method was validated for specificity, linearity, precision, accuracy, and repeatability. The method was linear in the range 10-60 $\mu\text{g/mL}$ per band. Study of puerarin was found 99.90% by this method. The method is simple, specific, precise, accurate and reproducible for estimation of puerarin in pueraria species and its formulations.

Keywords: Ayurveda, Herbal, HPTLC, *Pueraria tuberosa*, Puerarin, Standardization, Vaajikaran,

INTRODUCTION

Pueraria tuberosa (Roxb.ex Willd.) DC. (Family: Fabaceae) is a rasayana herb which is popularly known as 'Vidarikand' in Ayurvedic system of medicine. The tuber of *Pueraria* is sweet in taste and used in indigenous system of Indian medicine as tonic, aphrodisiac, antirheumatic, diuretic and galactagogue [1]. It is an important constituent of Ayurvedic medicines including Chywanprash, a popular tonic [2]. Puerarin is an important isoflavone-C-glucoside found in *P. lobata* (Willd.) Ohwi, *P. phaseoloides* (Roxb.) Benth., and *P. tuberosa*. The methods previously reported for estimation of puerarin include HPLC, liquid chromatography-mass spectrometry and Spectrofluorometry [3-7]. This study reported a HPTLC method for analysis of puerarin from ethanolic extract of tubers of *P. tuberosa*.

MATERIALS AND METHODS

Collection and Identification of Plant

Tubers of *P. tuberosa* were collected in the month of November from the forest surrounding the campus of Dr. H. S. Gour University, Sagar and were authenticated in Department of Botany, Dr. H. S. Gour University, Sagar and a herbarium deposited (PT: NSC/H /2008/02). The plant material was coarsely powdered using a mechanical grinder and stored in an airtight container.

Preparation of Ethanolic Extract

Tubers of *P. tuberosa* were first defatted with petroleum ether (60-80 °C). The defatted marc was extracted with

ethanol (95%) in a soxhlet apparatus. The solvent from ethanolic extract was evaporated under vacuum to yield the ethanolic extract, which was used for present pharmacological investigation.

Materials and Chemicals

Materials and Chemicals include ethanolic extract of tubers of *P. tuberosa*, marker compound puerarin, ethyl acetate, methanol, chloroform, toluene, and distilled water. All solvents were analytical grade. Equipments include aluminium backed precoated silica gel 60F₂₅₄ HPTLC plates (Merck KGaA, Germany), Whatman filter paper (No. 41), sample applicator: LINOMATE 5, Hamilton's glass syringe (100 μL), development chamber: Twin trough chamber (10 \times 10 or 20 \times 10 cm), Scanner: CAMAG SCANNER 3, UV Cabinet with short wave (254 nm) and long wave (366 nm) tubes, dipping chamber and plate dryer.

Quantization of Marker Compound Puerarin in Ethanol Extract

The ethanol extract of *P. tuberosa* was found the presence of marker compound puerarin, which was confirmed by the results of Co-TLC with standard puerarin Fig. (1). The ethanolic extract was taken for further analysis by HPTLC method for estimation and method development of marker compound in the ethanolic extract of crude drug for quality evaluation and standardization of crude drug.

Preparation of Standard Solution

The standard puerarin (10 mg) was accurately weighed and dissolved in 10 mL of HPLC grade methanol. Then the stock solution was filtered through Whatman filter paper and aliquots of 10 μL were taken for application.

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Preparation of Sample Solution

Ethanol extract (100 mg) of *P. tuberosa* was dissolved in 10 mL of ethanol in a volumetric flask. The stock solution of sample was filtered through Whatman filter paper and 10 μ L solution was taken for sample application.

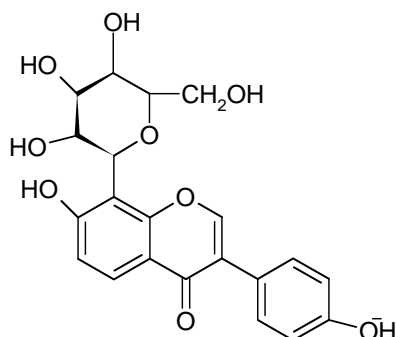


Fig. (1). Structure of Puerarin.

Preparation of Solvent System

The developed solvent system containing chloroform:

methanol (9:1) was used and prepared by mixing the solvents in desired ratio in the conical flask and shaken thoroughly. The solvent system was set aside for 15 minutes.

Preparation of Standard Curve of Puerarin

Calibration curve of standard puerarin was prepared by applying the different concentrations (10-50 μ g) of standard solution on HPTLC plates in triplicate using Camag Automated TLC Sampler 4, in the form of bands. The plates were dried at room temperature and were developed in a 10 \times 10 cm twin-trough glass chamber without chamber saturation to a distance of 8 cm. After the development, plates were dried at room temperature and scanned for densitometrically on a Camag scanner 3 using Camag TLC software (win CATS). The quantification was done at wavelength of maximum absorption at 254 nm. Peak area of standard compound was recorded and calibration curve was prepared by plotting peak area versus concentration of puerarin applied.

Chromatographic Procedure for Sample

10 mL of freshly prepared mobile phase consisting of specific solvent was poured in a 10 \times 10 cm twin through

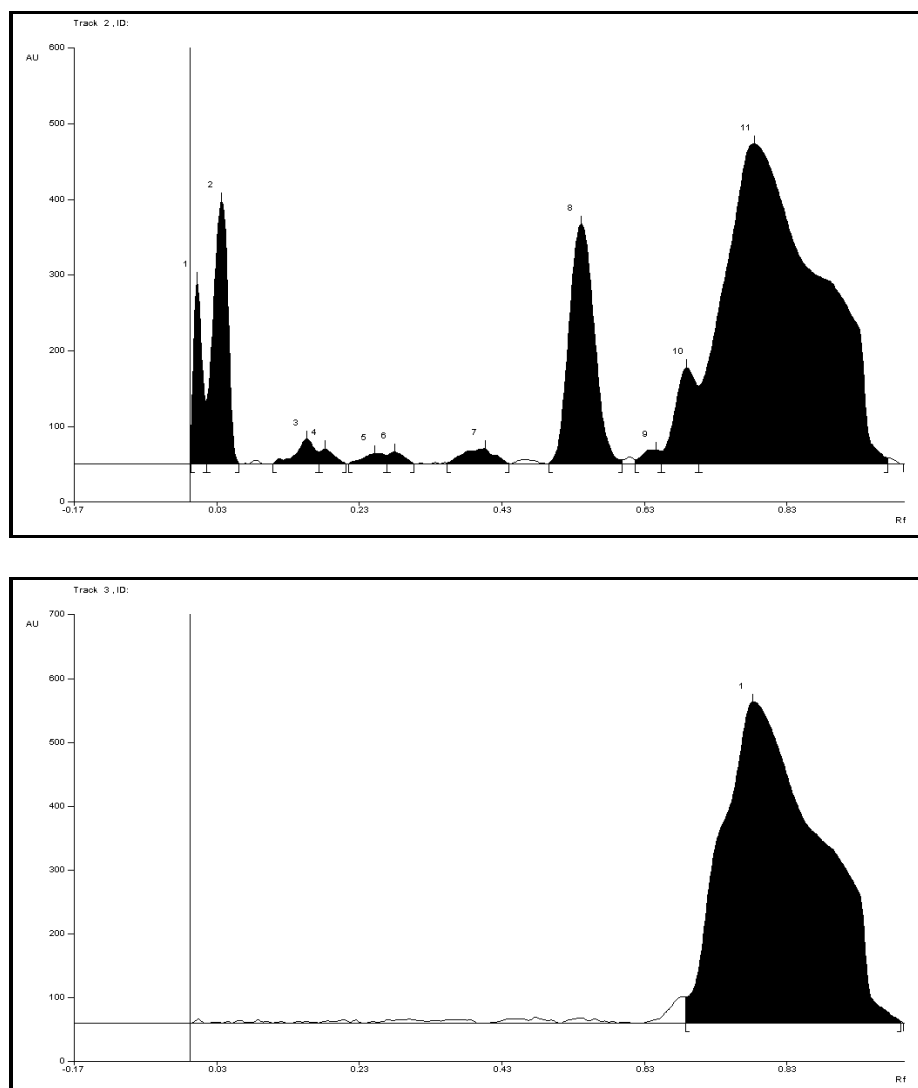


Fig. (2). HPTLC chromatogram of ethanolic extract of *Pueraria tuberosa* and puerarin at wavelength 254 nm.

Table 1. Validation parameters for Puerarin quantification by HPTLC method

Parameter	Result
Detection wavelength	254 nm
Linearity range	10-60 ($\mu\text{g/mL}$)
Precision (% RSD)	0.28
Repeatability (% RSD)	1.46
Regression equation ($y = mx + c$)	$Y = 948.44 x - 959.6$
Coefficient of correlation	$R^2 = 0.9942$
Precision (%RSD)	
Intra-day (n = 5)	0.64
Inter-day (n = 5)	0.42

Table 2. Recovery study of puerarin by HPTLC method

Sample No.	Amount of Puerarin Present in the Sample (μg)	Amount of Puerarin Added (μg)	Amount of Puerarin Found (μg)	Recovery (%)	Average % Recovery
1	42.1	20	61.94	99.74	99.90
2	42.1	30	72.12	100.02	
3	42.1	40	82.06	99.95	

glass chamber. The standard (10 μL) and extract sample solution (10 μL) in triplicate were applied on the plate using Camag Automated TLC Sampler in the form of bands. The length of the band was kept 6.0 mm and the distance between the two bands was kept at 8 mm. After drying at room temperature, the plate was inspected under UV light and scanned for densitometry chromatographic evaluation on a Camag scanner 3 using Camag TLC software (win CATS). The concentration of the marker compound puerarin in the ethanolic extract was determined using regression equation of standard curve of puerarin.

Analytical Method Validation

The method was validated for repeatability, precision and accuracy [8]. Repeatability of method was affirmed by multiple measurement (n = 5) of puerarin (200 ng per spot) after application on TLC plates under the same analytical procedure and laboratory conditions. The result was expressed as coefficient of variance (% RSD).

Precision of instrument was checked by repeated scanning (n = 5) of the same spot of puerarin (200 ng) and was expressed as % RSD. Variability of method was studied by analyzing aliquots of standard puerarin (100, 200, 300, 400, and 500 ng) on the same day (Intra day precision) and on different day (Inter day precision) and the result was expressed as % RSD.

Accuracy of the method was checked by adding known amount of puerarin (20, 30, and 40 $\mu\text{g/mL}$) in ethanolic extract solution (42.1 $\mu\text{g/mL}$) and estimated by method above.

The percentage recoveries were calculated on the basis of determination of the analyte added to a sample containing a known amount of puerarin. The percentage recovery as well as average percentage recovery was calculated.

RESULTS

Fingerprinting of ethanolic extract was performed using chloroform: methanol (9:1) as mobile phase. The plate was scanned at 254 nm. It showed eleven compounds in HPTLC chromatogram. The component in ethanolic extract of *P. tuberosa* tubers appeared at R_f values, 0.00, 0.02, 0.11, 0.18, 0.22, 0.27, 0.36, 0.50, 0.62, 0.66, and 0.71 and peak area %, 3.49, 8.5, 1.01, 0.46, 0.50, 0.41, 1.05, 13.20, 0.57, 4.51, and 66.29 respectively Fig. (2). Ethanol extract was also quantified for marker compound puerarin. The standard curve of puerarin was made with the help of HPTLC. The regression equation from standard curve was found $y = 948.44 x - 959.6$ and linearity range 10-60 ($\mu\text{g/mL}$) respectively. Recovery study of puerarin was found 99.90 % by this method. The concentration of puerarin in extract was found 0.42 (% w/w) (Tables 1 and 2).

CONCLUSION

The results of the study clearly demonstrate that the established HPTLC method could be beneficially used for the purpose of quantity evaluation of puerarin content. Validation confirmed this is an appropriate method for routine quality control of formulations containing puerarin.

CONFLICT OF INTEREST

Declared none.

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