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Quantification of puerarin from *Pueraria tuberosa* DC by using high performance liquid chromatography

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method has been developed for the determination of puerarin in root powder of *Pueraria tuberosa* DC. The analysis was done with Thermo Hypersil BDS C18 column (150mm×4.6 mm i.d., 5µm particle size) as stationary phase at a wavelength of 254 nm for detection and determination. The proposed HPLC method was validated for linearity, accuracy, precision and limit of quantitation. The validated HPLC method can be used for a routine quality control analysis of *Pueraria tuberosa* DC. and quantitative determination of puerarin from root powder of *Pueraria tuberosa* DC.

KEYWORDS

Pueraria tuberosa DC;
Puerarin;
HPLC.

INTRODUCTION

In Ayurveda, 'Vidari' is botanically equated to *Pueraria tuberosa* DC. of fabaceae family. Reported chemical constituents in *Pueraria tuberosa* DC. are puerarin[1], β-sitosterol[1], tuberosin[2]. *Pueraria tuberosa* is a large, perennial climber with very huge tuberous roots, used to treat many pharmacological activities of which antihepatotoxic activity[3] and anti-implantation activity in rats[4] are few to name.

Determination of puerarin in *Pueraria tuberosa* DC by HPTLC is reported[5]. Determination of puerarin in Chinese traditional medicinal preparation by HPLC is reported[6]. A simple, rapid, precise, and accurate HPLC method has been established for determination of puerarin in *Pueraria tuberosa* DC.

EXPERIMENTAL

Reagents and materials

Ammonium acetate and HPLC grade Methanol and Water were obtained from Qualigens Fine Chemicals, Mumbai, India. Standard puerarin was procured from Sigma-Aldrich Chemie (Steinheim, Germany).

Roots of *Pueraria tuberosa* DC. were collected from Thane, India and were authenticated by the National Botanical Research Institute (NBRI), Council of Scientific and Industrial Research, Lucknow, India. The authenticated herbarium is preserved in duplicate, one at NBRI and another at the place of research, i.e. Ramnarain Ruia College, Mumbai, India, for future reference.

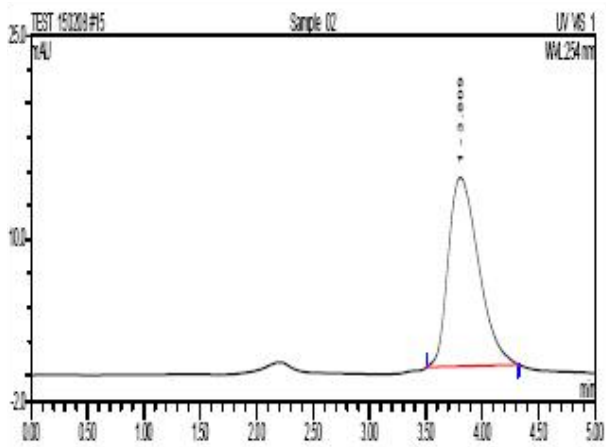

Figure 1 : HPLC chromatogram of plant

Standard and sample preparation

The stock solution (A) of puerarin (1000µg mL^{-1}) was prepared by dissolving 25mg of accurately weighed puerarin in minimum quantity of methanol and diluting with methanol upto the mark in a 25mL standard volumetric flask. Intermediate solution (B) of puerarin (100µg mL^{-1}) was prepared by transferring 1mL of stock solution (A) and diluting with mobile phase upto the mark in a 10mL volumetric flask. Intermediate solution (C) of puerarin (10µg mL^{-1}) was prepared by transferring 1mL of Intermediate solution (B) and diluting with mobile phase up to the mark in a 10mL volumetric flask. Different volumes of Intermediate solution (B) and Intermediate solution (C) were transferred to 10mL standard volumetric flasks and diluted upto the mark with the mobile phase, to provide a linearity range of 0.1 to 100.0µg mL^{-1}.

Roots of *Pueraria tuberosa* DC. were collected, washed, dried in the shade, and powdered. The powder was passed through an 80-mesh sieve and stored in an airtight container at room temperature. 10mg of the accurately weighed, dried whole plant powder was transferred to a 10mL standard volumetric flask and made upto the mark with the methanol. The solution was vortexed for five minutes 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. After filtration 2ml of above sample was transferred to 25ml standard volumetric flask and diluted upto the mark with the mobile phase. This sample solution was used for the assay.

TABLE 1 : Linearity data for puerarin *- of the equation y = mx + c, where y is peak area, m is the slope, x is the concentration and c is the intercept

Data	Puerarin
Linearity range µg mL^{-1}	0.1 to 100
Slope (m)*	2.6681
Intercept (c)*	-0.0753
Correlation coefficient (R)	0.9999
LOD µg mL^{-1}	0.05
LOQ µg mL^{-1}	0.1
Instrument Precision (RSD[%], n = 10)	0.21
Intraday Precision (RSD[%], n = 3)	0.17
Interday Precision (RSD[%], n =3)	0.18

Instruments

The HPLC analysis was carried out with an HPLC system (Jasco, Japan) equipped with PU-980 isocratic pump fitted with an AS-2057 auto sampler. The system controlling and data acquisition was performed through Borwin software.

Chromatography

Procedure

The analysis was performed with Thermo Hypersil BDS C_{18} column (150mm×4.6 mm i.d., 5µm particle size) reversed phase column. The mobile phase, mixture of 2mM Ammonium acetate and Methanol in the ratio (20:80 *v/v*) was filtered through 0.45µm membrane filter (Millipore) and degassed by sonication. Total run time was 5 minutes. Throughout the run flow rate of 0.5mL min^{-1} was maintained. The column effluent was monitored at 254nm with Jasco UV Visible detector (Model UV- 970) variable wavelength detector.

The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape for puerarin. A typical HPLC chromatogram of plant is shown in figure 1.

2mM Ammonium acetate preparation: Dissolve 154.16mg of ammonium acetate in water and make volume upto the mark in 1000ml standard flask with water.

System suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by injecting 20µL of the standard solution of puerarin (10µg

mL^{-1}) six times. The %RSD was found to be 0.76, which was acceptable as it is less than 2 %.

Linearity of detector response

For the evaluation of linearity eight different standard solutions (0.1, 0.2, 0.5, 1.0, 10.0, 50.0, 80.0 and 100.0μg mL^{-1}) of puerarin were used. In each case, 20μL of the solutions was injected. A linear relationship between the peak area and the concentration was observed for puerarin in the range of 0.1μg mL^{-1} to 100μg mL^{-1}. The experiment was performed thrice and the mean of the peak area was used for the calculation. The linearity data 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 puerarin were 0.05μg mL^{-1} and 0.1μg mL^{-1} respectively.

Assay

20μL of plant extract was injected and peak area of puerarin was measured using the linearity equation. The assay procedure described was repeated seven times starting from weighing of the plant powder. The retention time of puerarin was 3.80 minutes. The mean assay value of puerarin was found to be 2.08±0.06%.

Accuracy

The accuracy of the methods was established by performing recovery experiments by the standard addition method. The recovery of standard puerarin added to *Pueraria tuberosa* DC. Root powder was studied at two different levels, each being analysed in a manner similar to that described for the assay. The content of puerarin was quantified by the proposed method and the percentage recovery was calculated. The recovery obtained for puerarin was from 98 to 102, showing the reproducibility of the method was good. The average recovery was found to be 100.78% for puerarin.

RESULTS AND DISCUSSION

Of the different mobile phases investigated, 2mM Ammonium acetate: Methanol 20:80 (*v/v*), resolved puerarin (Rt = 3.80) very efficiently from the other components of the methanolic extract of *Pueraria tuberosa* DC. The response to puerarin was found to be linearly dependent on concentration in the range 0.1μg ml^{-1} to 100μg ml^{-1}, with correlation coeffient of 0.9999.

The variability of the method was studied by analyzing aliquots of the different concentrations of puerarin solutions on the same day (intra-day precision) and on different days (inter-day precision) and by instrument precision. The results were expressed as % RSD. The % RSD values were found to be less than 2%, indicating that the selected method is precise and reproducible. The mean recovery of puerarin was found to 100.78%, which indicates the accuracy of the method.

The robustness of the method was studied, during method development, by determining the effects of small variation, of mobile phase composition (±2%). No significant change in R_t or in response of puerarin was observed, indicating the robustness of the method.

CONCLUSION

The proposed method is simple, rapid, precise, and accurate which can be used for routine quality-control analysis of *Pueraria tuberosa* DC. root powder and for quantitative determination of puerarin from root powder.

REFERENCES

[1] S.P.Bhutani, S.S.Chibber, T.R.Seshadri; Indian J.Chem., **7**, 210- 212 **(1969)**.

[2] A.V.K.Prasad, A.Singh, R.S.Kapil, S.P.Popli; Indian J.Chem.B, **23**, 1165-1167 **(1984)**.

[3] F.L.Hsu, I.M.Liu, D.H.Kuo, W.C.Chen, H.C.Su, J.T.Cheng; J.Nat.Prod., **66**, 788-792 **(2003)**.

[4] S.Shukla, S.Jonathan, A.Sharma; Phytother.Res., **10**, 608-609 **(1996)**.

[5] H.P.Mhaske, V.V.Vaidya; Journal of Planar Chromatography, **21**, 65-67 **(2008)**.

[6] Yu Bing-Sheng, Xiao-Pin Yan; Journal of Pharmaceutical and Biomedical Analysis, **30**, 843-849 **(2002)**.