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SPECTROFLUORIMETRIC ESTIMATION OF PUERARIN IN *PUERARIA TUBEROSA*

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Pueraria tuberosa (Roxb. ex. Willd.) DC. (Fabaceae) is the important plant used in Indian medicine, commonly known as Vidarikand. The plant is described as rasayana and tonic in Ayurvedic Pharmacopoeia of India (1). It is an extensive perennial climber, growing throughout tropical parts of India, mostly in moist regions, monsoon-forests and coastal tracts (2). The tuber of pueraria is sweet in taste and used in indigenous system of Indian medicine as tonic, aphrodisiac, antirheumatic, diuretic and galactogogue (3). It is an important constituent of Ayurvedic medicines including Chywanprash, a popular tonic (4). Phytoconstituents like: puerarin, daidzein, β-sitostreol, stigmasterol, puerarone and coumestan, isoflavone C-glycoside-4,6-diacetyl-puerarin, pterocarpintuberosin, puetuberosanol and hydroxytuberosone have been isolated and characterized from the species (5).

Puerarin is an important isoflavone-C-glucoside also found in a number of plants like *Radix puerariae* (6), *Pueraria lobata* (7) and *Pueraria phaseoloides* (8). Puerarin and its preparations (injections) are official in Chinese Pharmacopeia (9). Its pharmacological activities like: anticancer (10), hepatoprotective (11), estrogenic (12), antioxidant (13), antidiabetic (14), neuroprotective (15) and in learning-memory disorder (16) have been reported.

The methods previously reported for estimation of puerarin include HPLC, HPTLC and liquid chromatography-mass spectrometry (17–19). The above methods for estimation of puerarin are time consuming and costly. Methanolic solution of puerarin showed intense blue fluorescence when observed under UV light (366 nm); therefore, it was thought to develop a more sensitive and simple spectrofluorometric method for the estimation of puerarin. Using standard sample preparation procedures and fluroscence spectrometry detection, we established a rapid and simple assay for puerarin quantitation in any herbal formulation and plasma samples. This analytical method achieves the highest sensitivity of any method reported to date, enabling improved characterization of puerarin pharmacokinetics. The methods give leads to scientist to use spectroflurometry to estimate fluorescence compound.

EXPERIMENTAL

Materials and methods

The spectrofluorimetric study was carried out with a Shimadzu RF 5301 PC spectrofluorimeter; the light source used was a xenon 150 W lamp with an optical system composed of two automatic monochromators, one for excitation and the other for emission of a mesh type to enable a suitably wide selection of excitation and emission wavelengths. A quartz cell was used. The detection system comprised of R 450-01 photomultiplier, which transformed the fluorescent radiation emitted by the fluorescent solution in the cell into an electrical signal.

Preparation of extracts:

Tubers of *Pueraria tuberosa* (PT) were powdered and extracted with methanol for 15 min.

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Preliminary analysis

A preliminary analysis was carried out to determine the wavelength at which maximum intensity is exhibited by pure puerarin. For this purpose, 1000 ng/mL sample of pure puerarin was prepared in methanol. This solution was scanned spectrofluorimetrically to obtain the excitation and emission wavelengths. The λ_{max} shown by puerarin had an excitation at 246 nm and an emission at 483 nm.

Table 1. Optical characteristics and analytical data.

Parameters	Results
Excitation wave length	246
Emission wave length	483
Linearity range (ng/mL)	100-1000 ng/mL
Regression equation	y = 0.3625x -1
Slope	0.3625
Intercept	1
Coefficient of determination (r^2)	0.9988
Limit of detection (ng/mL)	38.62
Limit of quantification	86.81
Accuracy %	99.91

Preliminary spectrofluorimetric screening of the methanolic extract for testing the wavelength range

The methanolic extract of PT was scanned in the spectrofluorimeter in concentrations of 0.1 µg/mL and 0.01 µg/mL. The scanning was performed from 200 nm to 700 nm and absorption maxima were found at 246 nm and 483 nm. This wavelengths corresponded to λ_{max} of puerarin. No other absorption peaks were detected in the range screened. Scanning between 246 nm and 483 nm did not exhibit any other peak, thus the range selected for analysis was expected to screen only puerarin in the test sample.

Preparation of standard curve

Standard curve of puerarin was prepared in methanol. First of all, stock solution containing 1000 µg/mL of puerarin was prepared in methanol. Then this stock solution was used for preparing

Table 2. Data of calibration curve for puerarin.

Concentration of puerarin (ng/mL)	Relative fluorescence intensity	% RSD
100	38	0.45
200	74	0.67
300	106	0.56
400	140	0.94
500	182	1.2
600	210	0.86
700	254	0.42
800	294	1.3
900	321	1.1
1000	365	0.96

Table 3. Validation of the spectrofluorimetric method.

Sample No.	Calculated amount of puerarin in the extract (ng/mL)#	Calculated amount of puerarin in the standard solution (ng/mL)#	Total amount of puerarin in mixture (ng/mL)	Amount analyzed (ng/mL)	Percentage recovery (%)
1	169.43	100	269.43	268.77 ± 0.46	99.74 ± 0.17
2	238.86	100	338.86	449.20 ± 0.29	100.07 ± .06
3	847.05	100	947.05	946.59 ± 0.47	99.94 ±. 04

#As calculated from the standard curve. All values are the mean ± SD (n = 3). Excitation (λ_{max}: 246 nm), emission (λ_{max}: 483 nm)

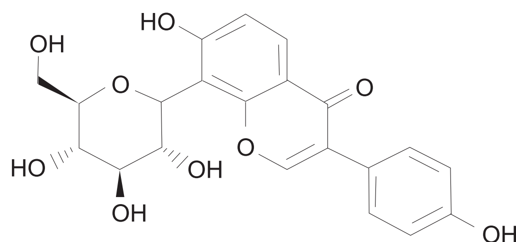

Figure 1. Chemical structure of puerarin (7-hydroxy-3-(4-hydroxyphenyl)-8-[(3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-4-one)

required dilutions containing 100–1000 ng/mL of puerarin. These samples were analyzed in the spectrofluorimeter against solvent blank (methanol). The wavelength and intensity for each sample was recorded and standard curve was prepared between concentration and intensity of fluorescence. The equation of line for the standard curve for puerarin was y = 0.3625x – 1 and correlation coeffcient (r^2) value was 0.9988.

Ten milligrams of methanol extract was weighed accurately and dissolved in 10 mL of methanol with vigorous shaking. It was filtered and volume made to 100 mL in distilled water. This solutions was analyzed in the spectrofluorimeter and intensity of fluorescence was recorded. Further, concentration of standard in the extract samples was determined from the standard curve.

Determination of puerarin concentration in methanolic extract

Ten milligrams of the methanolic extract was weighed accurately and dissolved in 10 mL of methanol with vigorous shaking. It was then filtered and the volume made up to 100 mL with methanol.

This solution was analyzed in the spectrofluorimeter and intensity of fluorescence was recorded. The concentration of puerarin in the extract samples was determined from their standard curves.

Analytical method validation (20)

Linearity

Standard solutions (100 ng/mL to 1000 ng/mL) were prepared in methanol and the intensity of fluorescence was recorded in the spectrofluorimeter. The standard curve was prepared by plotting the concentration as the abscissa *versus* the intensity of fluorescence as the ordinate. A linear dependence of intensity on concentration was observed over the entire concentration range tested.

Precision and accuracy

The precision of the method was checked using standard solutions of puerarin and adding the methanolic extract solution of different concentration prepared by appropriate dilutions with methanol. The solutions were analyzed in a spectrofluorimeter at 246 nm and 483 nm (excitation and emission wavelengths) for puerarin and the intensities were recorded. The corresponding concentrations were extrapolated from the standard curve. The entire procedure was repeated three times for each dilution and the readings were expressed as the mean ± SD (n = 3). Then, 100 ng/mL solutions of puerarin were prepared by appropriate dilutions and analyzed spectrofluorimetrically. The concentrations of puerarin were calculated for the sample. This sample of known concentration was added in an equal volume (1 mL) to all the previous dilutions, and analyzed to see whether the observed concentrations correspond to the theoretical concentrations from the standard curve. The % recoveries were calculated on the basis of determination of the analyte added to a sample containing a known amount of puerarin (Table 3).

RESULTS AND DISCUSSION

Standard curves for puerarin were prepared at excitation and emission wavelengths of 246 nm and 483 nm, respectively, using a spectrofluorimeter. The plots of concentration *versus* intensity exhibited a linear relationship. The equation of the straight line for puerarin was y = 0.3625x – 1. A methanolic extract of PT was also analyzed at the same excitation and emission wavelengths. The puerarin content calculated from the standard curve was found to be 0.2%, respectively. Thus, a simple analytical method was developed for determination of concentrations of puerarin in PT. The developed method

was validated for linearity, reproducibility and accuracy. The linearity was found to be in the range of 100–1000 ng/mL. The correlation coefficients (r^2) were 0.9988, respectively, indicating good linearity between the fluorescence intensity and concentration. Scanning of the samples three times allowed the precision of the method to be checked. The reproducibility and accuracy of the method was checked by carrying out recovery studies. A sample of known concentration of standard solution of puerarin was added in equal volume to the various dilutions of the extract and analyzed spectrofluorimetrically to see whether the observed concentration obtained corresponded to the theoretical concentration obtained from the standard curve. The percentage recovery of puerarin was found to be in the range of 99.91%. Hence, the developed spectrofluorimetric procedure is a quick and reliable method for the quantitative estimation of puerarin in raw material, processed powder and in herbal preparations containing *Pueraria tuberosa.*

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REFERENCES

1. Anonym: The Ayurvedic Pharmacopoeia of India, Part I, Vol. V, p. 193, Ministry of Health and Family Welfare, New Delhi 2006.
2. Chopra R.N., Nayar S.L., Chopra I.C.: Glossary of Indian Medicinal Plants. CSIR, New Delhi 1956.
3. Kirtikar K.R., Basu B.D.: Indian Medicinal Plants. p. 792, Lalit Mohan Basu, Allahabad 1935.
4. Anonym.:Ayurvedic Formulary of India. Government of India, Ministry of Health and Family Planning, Department of Health, New Delhi 1978.
5. Devaiah K.M., Venkatasubramanian P: Curr. Sci. 94, 1306 (2008).
6. Yeung D.K., Leung S.W., Xu Y.C., Vanhoutte P.M., Man R.Y.: Eur. J. Pharmacol. 552, 105 (2006).
7. Chen G., Zhang J., Jiannong Y.: J. Chromatogr. A 923, 255 (2001).
8. Kintzios S., Makri O., Pistola E., Matakiadis T., Shi H.P., Economou A.: Biotechnol. Lett. 13, 1057 (2004).
9. Anonym.:Pharmacopoeia of the People's Republic of China. p. 707, Chinese Pharmacopoeia Commision, People's Medical Publishing House, Beijing 2005.
10. Hien T.T., Kim H.G., Han E.H., Jeong K.G.: Mol. Nutr. Food Res. 54, 918 (2010).
11. Hwang Y.P., Choi C.Y., Chung Y.C., Jeon S.S., Jeong H.G.: Arch. Pharm. Res. 10, 1309 (2007).
12. Malaivijitnond S., Tungmunnithum D., Gittarasanee S., Kawin K, Limjunyawong N.: Fitoterapia 81, 569 (2010).
13. Bebrevska L., Foubert K., Hermans N., et al.: J. Ethnopharmacol. 127, 112 (2010).
14. Li Q., Xiao Y., Gong H., Shen D., Zhu F., et al.: Nat. Med. J. India 22, 9 (2009).
15. Chang Y., Hsieh C.Y., Peng Z.A., Yen T.L., Hsiao G., et al.: J Biomed Sci. 16, 9 (2009).
16. Wu H.Q., Guo H.N., Wang H.Q., Chang M.Z., Zhang G.L., Zhao Y.X.: Chin. J. Integr. Med. 15, 54 (2009).
17. Congbing F., Xiaochun W., Changjun J., Tan H.R.et al.: J. Planar Chromatogr. 19, 348 (2006).
18. Ren F., Jing Q., Shen Y., Ma H., Cui J.: J. Pharm. Biomed. Anal. 41, 549 (2006).
19. Ching C.L., Chuan I.W., Shuenn J.S., J. Sep. Sci. 28, 1785 (2005).
20. Anonym: ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Geneva 2005.

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