



Trade Science Inc.

March 2010

ISSN : 0974-7419

Volume 9 Issue 1

# Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 9(1) 2010 [35-38]

## Liquid chromatography-tandem mass spectrometric method for determination of puerarin from *Pueraria tuberosa*

Harshad P.Mhaske\*, Vikas V.Vaidya, Shveta H.Mhaske, Pramod N.Pabrekar, Maharudra B.Kekare  
Department of Chemistry, Ramnarain Ruia College, Matunga, Mumbai-400 019, (INDIA)

E-mail : harshadmhaske1982@gmail.com

Received: 5<sup>th</sup> January, 2010 ; Accepted: 15<sup>th</sup> January, 2010

### ABSTRACT

*Pueraria tuberosa* is a commonly used Chinese herbal medicine, which contains a series of isoflavones as its chief pharmacologically active constituents. Using puerarin as marker, a rapid, reproducible and efficient liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed to quantify puerarin from *Pueraria tuberosa*. The analyte and Internal Standard (IS) were chromatographed on a Hypurity C<sub>18</sub> (50mm×4.6mm i.d., 5μ particle size) column using 10μL injection volume with a run time of 2 min. The precursor and product ion of analyte and IS were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring mode (MRM) with positive polarity. The proposed method was validated over the range of 25μg/ml to 750μg/ml. The proposed LC-MS/MS method was validated for linearity, accuracy, precision and limit of quantitation. © 2010 Trade Science Inc. - INDIA

### KEYWORDS

*Pueraria tuberosa*;  
Puerarin;  
LC-MS/MS;  
Multiple reaction monitoring.

### INTRODUCTION

In Ayurveda, *Pueraria tuberosa* is known as 'Vidari' whose tuber is used for wide variety of ailments. It is used as aphrodisiac, tonic, galactagogue, diuretic, antidiabetic and alterative<sup>[1]</sup>. The root is given in demulcent and refrigerant in fever. It is used in rheumatism and also to reduce swelling of joints. *Pueraria tuberosa* belongs to the Family *Fabaceae*. Reported chemical constituents in *Pueraria tuberosa* are puerarin<sup>[2]</sup>, β-sitosterol<sup>[2]</sup>, tuberosin<sup>[3]</sup>. *Pueraria tuberosa* is a large, perennial climber with very huge tuberous roots, used to treat many pharmacological activities of which antihepatotoxic activity<sup>[4]</sup> and anti-implantation activity in rats<sup>[5]</sup> are few to name. The structure of puerarin is shown in figure 1.

Determination of puerarin in *Pueraria tuberosa* by HPTLC is reported<sup>[6]</sup>. Determination of puerarin in Chinese traditional medicinal preparation by HPLC is reported<sup>[7]</sup>. The aim of this study was to develop a selective, rapid, precise, and accurate method for determination of puerarin in *Pueraria tuberosa*.

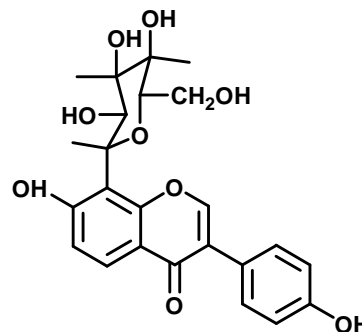


Figure 1 : Structure of puerarin

## Full Paper

### EXPERIMENTAL

Standard puerarin and azithromycin (IS) were procured from Sigma-Aldrich Chemie (Steinheim, Germany). AR grade Ammonium acetate was procured from Quligens Ltd. (Mumbai, India). HPLC grade Methanol was purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Purified water was obtained from Milli Q A10 gradient water purification system (Millipore, Bangalore, India).

Roots of *Pueraria tuberosa* were collected from Thane, India and were authenticated by the National Botanical Research Institute (NBRI), Council of Scientific and Industrial Research, Lucknow, India.

#### Standard and sample preparation

The stock solution (A) of puerarin ( $1000\mu\text{g mL}^{-1}$ ) and azithromycin ( $10\mu\text{g mL}^{-1}$ ) was separately prepared in Methanol. Azithromycin was used as Internal Standard (IS). Working solutions of puerarin in the required concentration range were prepared by appropriate dilution of the stock solutions in Methanol: Water (50:50 v/v). All the solutions were stored at  $2-8^{\circ}\text{C}$  and brought to room temperature before use.  $10\mu\text{L}$  of the IS solution was added uniformly while preparation of the linearity range of  $25.0\mu\text{g mL}^{-1}$  to  $750.0\mu\text{g mL}^{-1}$ .

Roots of *Pueraria tuberosa* 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. 5 mg 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 1ml of above sample was transferred to 25ml standard volumetric flask and diluted upto the mark with the Methanol: Water (50:50 v/v). This sample solution was used for the assay.

#### Liquid chromatography and mass spectrometric conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary LC-10AD prominence pump, autosampler (SIL-HTc) and solvent degasser (DGU-14) were used for the analysis. For separation, the samples were analysed without any guard column on Hypersil Hypurity  $\text{C}_{18}$  ( $50\text{mm}\times 4.6\text{mm}$  i.d.,  $5\mu$  particle size) analytical column of Thermo (I) Pvt. Ltd. (India). The flow rate of the mobile phase under isocratic condition was kept at  $0.4\text{ml/min}$ . The autosampler temperature was set at  $4^{\circ}\text{C}$  and the injection volume was  $10\mu\text{L}$ . Column oven temperature was set at  $40^{\circ}\text{C}$ . The mobile phase consisted of  $2\text{mMol/L}$  ammonium acetate (pH 3.5) and Methanol (20:80 v/v). The total run time was 2.0 min. Detection of analyte and IS was performed on a triple Quadrupole mass spectrometer, API-3000, (MDS SCIEX, Toronto, Canada) equipped with Turbo ion spray ionization source and operating in positive ion mode. Analyst software version 1.4.1 was used to control all parameters of LC and MS. Quantitation was performed using multiple reaction monitoring (MRM) mode to study parent  $\rightarrow$  product ion (m/z) transitions for Puerarin (417.1/297.0) and internal standard azithromycin (749.5/591.3). The typical fragmentation pattern of Puerarin is shown in figure 2.

Source dependent parameters optimized were: Gas 1 (Nebuliser gas): 12 psi; Gas 2 (Heater gas flow):  $8000\text{cc/min}$ ; Temp (TEM):  $450^{\circ}\text{C}$ ; Ion spray voltage: 5500 volts. Compound dependent parameters were like: Declustering potential (DP), Entrance potential (EP),

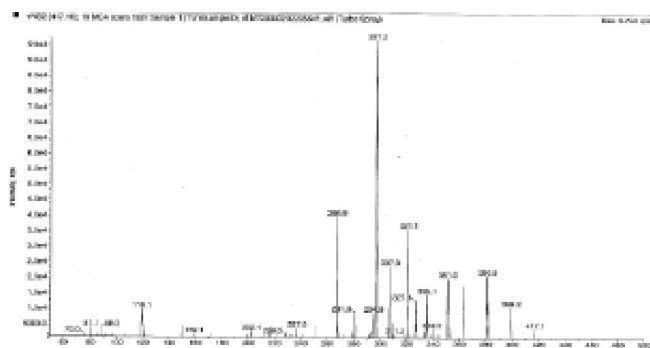


Figure 2 : Fragmentation pattern of puerarin

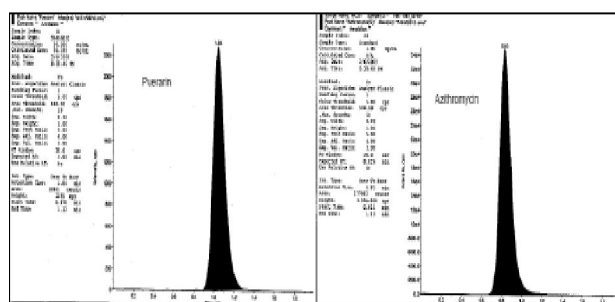


Figure 3 : Chromatogram of puerarin and IS

Focusing Potential (FP), Collision Energy (CE) and Cell Exit Potential (CEP) were 92 V, 10 V, 350 V, 30 V and 6 V for puerarin while for azithromycin were 45 V, 14 V, 370 V, 40 V and 15 V respectively. Nitrogen was used as Collision activated dissociation (CAD) gas and was set at 6psi. Quadrapole 1 and Quadropole 3 both were maintained at unit resolution and dwell time was set at 400msec for both.

### Analytical data processing

Chromatographic data was collected and integrated using Analyst software version 1.4.1. Peak area ratio of Puerarin/ IS was utilized for the construction of calibration curve. Weighing of  $1/x$  (linear regression analysis where  $x$  is the analyte concentration) was used for curve fitting. Concentration of puerarin in plant sample was calculated from the equation ( $y=mx+c$ ), where  $y$  is the peak area ratio.

### Method validation

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by injecting 10 $\mu$ L of the standard solution of puerarin (375 $\mu$ g mL<sup>-1</sup>) six times. The %RSD was found to be 1.76, which was acceptable as it is less than 5 %.

The linearity of the method was determined by analysis of standard plots associated with a 9-point standard calibration curve. Three linearity curves containing nine non-zero concentration (25.0, 50.0, 62.5, 75.0, 150.0, 375.0, 500.0, 600.0 and 750.0 $\mu$ g mL<sup>-1</sup>) were analyzed. Calibration curves of peak area ratio versus concentration were drawn. In each case, 10 $\mu$ L of the solutions were injected. A linear relationship between

**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 $\mu$ g mL <sup>-1</sup>	25.0 to 750.0
Slope (m)*	0.00260
Intercept (c)*	0.0088
Correlation coefficient (R)	0.9994
LOD $\mu$ g mL <sup>-1</sup>	5
LOQ $\mu$ g mL <sup>-1</sup>	25
Instrument Precision (RSD[%], n = 10)	1.21
Intraday Precision (RSD[%], n = 3)	1.17
Interday Precision (RSD[%], n =3)	1.18

the peak area ratio and the concentration was observed for puerarin in the range of 25.0 $\mu$ g mL<sup>-1</sup> to 750.0 $\mu$ g mL<sup>-1</sup>. The experiment was performed thrice and the mean of the peak area was used for the calculation. Typical Chromatograms of Puerarin and IS are shown in figure 3.

The signal-to-noise ratio of 3:1 and 5:1 was used to establish LOD and LOQ respectively. The LOD and LOQ of puerarin were 5.0 $\mu$ g mL<sup>-1</sup> and 25.0 $\mu$ g mL<sup>-1</sup> respectively. The linearity data is given in TABLE 1.

10 $\mu$ L 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 and IS were 1.04 min and 0.83 min respectively. The mean assay value of puerarin was found to be 2.22%.

Stock solution of puerarin and azithromycin were stable at room temperature for 24 hours and at 2-8°C for 32 days in Methanol.

## RESULTS AND DISCUSSION

Chromatographic analysis of analyte and internal standard was initiated under isocratic condition with an aim to develop a simple separation process with short run time. Separation was tried using various combinations of Methanol and buffer with varying contents of each component on variety of columns like C<sub>8</sub> and Betasil C<sub>18</sub> to identify the optimal mobile phase that produce the best sensitivity, efficiency and peak shape. Use of buffer of pH-3.5 helped in achieving good response for MS detection operating in positive mode. Thus the mobile phase consisting of 2mM Ammonium acetate with pH adjusted to 3.5 with formic acid: Methanol 20:80 v/v was found suitable for analyte and internal standard. High content of Methanol (80%) in Mobile phase helped in eluting the analyte and internal standard within 2 minutes at flow rate of 0.4ml/min. Hypersil Hypurity C18 (50mm $\times$ 4.6mm i.d., 5 $\mu$  particle size) column gave good peak shape and response even at LOQ level. For analyte and internal standard low injection volume of 10 $\mu$ l reduced overloading of column with analyte there by ensuring more number of analyses on the same column.

The response to puerarin was found to be linearly

## Full Paper

dependent on concentration in the range  $25\mu\text{g ml}^{-1}$  to  $750\mu\text{g ml}^{-1}$ , with correlation coefficient of 0.9994. 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 5%, indicating that the selected method is precise and reproducible.

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

## CONCLUSION

The developed LC-MS/MS method is selective, rugged, rapid, precise, and accurate which can be used for quantitative determination of puerarin from *Pueraria tuberosa* root powder.

## REFERENCES

- [1] R.N.Chopra, S.L.Nayar, I.C.Chopra; 'Glossary of Indian Medicinal Plants', Council of Scientific & Industrial Research; New Delhi, (1956).
- [2] S.P.Bhutani, S.S.Chibber, T.R.Seshadri; Indian J.Chem., **7**, 210- 212 (1969).
- [3] A.V.K.Prasad, A.Singh, R.S.Kapil, S.P.Popli; Indian J.Chem.B, **23**, 1165-1167 (1984).
- [4] 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).
- [5] S.Shukla, S.Jonathan, A.Sharma; Phytother Res., **10**, 608-609 (1996).
- [6] H.P.Mhaske, V.V.Vaidya; Asian J.Chem., **21**, 3449-3454 (2009).
- [7] Yu Bing-Sheng, Xiao-Pin Yan; Journal of Pharmaceutical and Biomedical Analysis, **30**, 843-849 (2002).