Ancient science of life vol: XXIII (3) January, February, March – 2004 Pages 22 - 25

DETERMINATION OF PUERARIN FROM PUERARIA TUBEROSA DC BY HPLC

Sateesh Kumar Chauhan, Brijpal Singh and Sudhakar Agrawal
R&D Centre, Indian Herbs,
Saharanpur – 247 001 (UP) India

Received : 14.5.2003 Accepted: 2.1.2004

ABSTRACT: A reverse phase High pressure liquid chromatography (HPLC) method to determine puerarin in Pueraria tuberose was developed and described. The method involves separation of active compound using mobile phase consisting of acetonitrile: water: orthophosporic acid in the ratio of 32:67:01 and detecting the chromatogram at 280 nm using photodiode array detector. The sensitivity of the method was observed as 1.0 mg and linearity was observed between 1.0 mg to 8.0mg. The puerarin content of 3.02% w/w was found in crude P.*tuberosa.* The proposed method in precise, sensitive, reproducible and easy to perform which can be used for detection, monitoring and quantification of puerarin in P. *tuberose.*

INTRODUCTION

The tuberous roots of Pueraria tuberose DC (Leguminosae) are used in medicine aphrodisiac, tonic, galactogogue, diuretic, emetic and alternative (1-3). They are consumed as supplementary food and for birth control by certain Indian tribes. The tubers have been reported to contain sterols (4), isoflavone, puerarin can be used for assessing the right quality of the tuber, quantitative estimation of which is very important of its standardization. The monograph of P. *tuberose* in Ayurvedic Pharmacopoea of India (6) provides just physico-chemical characteristics and no instrumental methods for quantification of active ingredients have been provided. The extensive literature survey indicated that lots of work have been performed on its chemistry and biological effects but no methods for its analytical standardization with reference to puerarin were found. An attempt has been made here for estimating puerarin content which is quick, simple, accurate and reproducible.

EXPERIMENTAL

Plant material

The fresh tubers of *Pureria tuberose* DC were collected locally form Himalyan foothills and identified by our botanist. The voucher specimen are preserved in the herbarium of our R&D Centre.

Extraction

The tubers were cut into small pieces and dried under shade and around 10gm were powered to pass through 40 mesh S.S. sieve. 1 gm from it was accurately weight in a 100ml conical flask and extracted with 15ml aqueous methanol (1:1) over steam water bath. The contents were boiled for 1-2 min. and filtered using laboratory grade filter paper in hot condition in to a separation conical flask of 250ml. The extraction was repeated four more time in a similar way and the filtrate obtained after each extraction were pooled and transferred to 100ml volumetric flask. The volume was made up to the mark with methanol. This filtered solution was used further for HPLC analysis.

HPLC Conditions

Instrumentation: A M/s Water's HPLC System consisting of a rheodyne 7125 injector, 996 photodiode array deterctor, 510 chromatographic pump and millennium software v 2.10.

Standard preparation

A 0.40 mg/ml solution of puerarin reference standard was prepared in methanol which was further diluted with methanol which was further diluted with methanol to yield the final concentration of 0.05, 0.10, 0.15 and 0.20 mg/ml.

Procedure

The test sample was filtered through 13 mm PVDF 0.45 micron syringe filter and 20 µ1 each of test sample and filter different concentrations of standard puerarin were injected to HPLC using nova pak C-18 reversed phase column.

The mobile phase consisting of acetonitrile: water: orthophosphoric acid – 32:67:01 was degassed using sonicator and used with a flow rate of 0.8 ml/min. The amount of puerarin in test sample was determined form the linear regression equation of calibration graph plotted between concentration and area. The equation obtained for puerarin was found to be $y=1227216x+24532$ with a correlation coefficient of 0.9999 where x is the concentration of the compound analysed in mg/ml and y is the response in peak area.

Method validation and recovery studies

A varying known amount of puerarin (1, 2 and 5mg) were added to about one gram of fine powdered test sample (40 mesh) in which the contents of puerarin had been estimated previously by proposed method. The samples were extracted and analysed separately as per the procedure mentioned above. The contents of puerarin were quantified using proposed method and percentage recovery calculated. The results are provided inTable-1.

RESULTS AND DISCUSSION

Standardisation of herbal medicines and plant ingredients with reference to their active/marker compound is the need of the time. Development of sophisticated chromatographic equipments has helped to develop such analytical methods. We have tried to standardize P.tuberosa using HPLC where we attemped to quantify one of its active compound, puerarin.

After taking several trials for developing suitable mobile phase, we observed that the mobile phase consisting of acetonitrile: water: orthophosphoric acid in the ratio of 32:67:01 by volume provided better separation and resolution of peaks. Using the proposed method, the retention time of puerarin was bout 7.5 min. The chromatogram of test sample showing the peak of puerarin is shown in Figure-1.

The calibration curve for puerarin was linear in the range of 1.0 to 8.0 mg. Further a correlation coefficient 0.9999 indicated good linearity between concentration and area. The method provides good resolution and separation of puerarin which allows reliable quantification. The average amount of puerarin in crude P.tuberosa was found to be 3.02%. Table -1 indicates an average recovery of 99.83 ±0.24%, which shows the reliability and suitability of the mothod. The proposed HPLC method is rapid, simple and accurate for quantitive monitoring of puerarin in P. tuberose and can easily be used for standardization of the plant material with respect to active principle.

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Table – 1

Method validation and recovery of puerarin from puerarin tuberose

Sl. No	**Details of Sample**	**Amount of sample taken (mg)**	**Amount of puerarin in A (mg)**	**Amount of puerarin added to A (mg)**	**Total puerarin taken B+C (mg)**	**Total puerarin found (mg)**	**% of Recovery $\frac{E}{D}$x100**
		(A)	**(B)**	**(C)**	**(D)**	**(E)**	
1.	*Pueraria tuberosa*	1025	30.95	1.00	31.95	31.88	99.78
2.	*Pueraria tuberosa*	1050	31.71	2.00	33.71	33.74	100.09
3.	*Pueraria tuberosa*	1022	30.86	5.00	35.86	35.72	99.61

Average percentage recovery = 99.83 ± 0.24%

Fig - 1 HPLC chromatogram of P. truberosa.