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Development and validation of HPLC method for Luteolin-7-glucoside in Chrysanthemum flower capsules

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Introduction

"Chrysanthemum flower capsules", developed from Chrysanthemum flower extract, is a herbal nutraceuticals for sedative effect. This product has passed efficacy and safety evaluation in animal testing. Chrysanthemum flower (*Chrysanthemum indicum* L), a herb of the Compositae family, is widely distributed in China and well-known with small yellow flowers. The aerial parts (stems, leaves and flowers) of *C. indicum* has been used as traditional medicine to treat vertigo, hypertensive symptoms and several infectious diseases such as pneumonia, colitis, stomatitis, carbuncle and fever. Its flowers are also commonly used as tea to treat some eye diseases in Chinese traditional medicine.[1] Chrysanthemum flower is known to contain several classes of biologically active compounds including essential oils, terpenoids, flavonoids, and phenolic acids. Among the nonvolatile oil, the major active components include flavonoids of luteolin-7-O-β-D-glucoside and linarin, as well as phenolic acids such as chlorogenic acid, 3,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid.[2] In this study luteolin-7-glucoside was chosen as a marker substance because of its health benefits.[3] The objective of this research is to develop and validate the analytical method of Luteolin-7-glucoside for quality control of this product. The chemical structures of luteolin-7-glucoside are shown in Fig. 1.

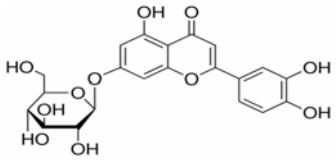

Figure1. Chemical Structures of Luteolin-7-glucoside

Methods

A. Reagents and samples

Luteolin-7-glucoside was purchased from Sigma-Aldrich, Germany. Acetonitrile and glacial acetic acid were HPLC grade from Lab-Scan, Thailand. All the water used in this study was Ultrapure, obtained from a Milli-Q RO system (Milipore Corporation, France). The Chrysanthemum flower capsules were developed in our research from Chrysanthemum extract.

B. Preparation of sample solution

The granules 150 mg was weighted and extracted with 30 ml methanol by sonicator for 15 minutes. The solution was filtered through a Whatman No.1. The filtrate was evaporated to less than 10 ml, then transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol. After filtering through a 0.2 µm syringe filter, the final sample was injected directly.

C. Preparation of standard solution

Luteolin-7-glucoside (10 mg) was dissolved with methanol (10 ml), to get stock solution containing 1000 µg/ml of Luteolin-7-glucoside.

The stock solutions were diluted to create the five-point standard curves of luteolin-7-glucoside using concentration at 25-200 µg/ml.

D. Instrumentation and chromatographic conditions

The analytical method of luteolin-7-glucoside was performed on a Waters Alliance e2695 LC system connected with a Waters model 2996 photodiode-array detector. Data collection and processing were carried out using an Empower workstation. The optimum HPLC system was comprised of a C18 reverse phase column (Luna C18, 250x4.6 mm i.d., 5 µm particle size). The gradient was eluted with acetonitrile and 1%acetic acid at a flow rate of 0.6 ml/min and PDA detection at 343.8 nm. The mobile phase consisted acetonitrile and 1%acetic acid and all solutions were degassed and filtered through a 0.20 µm pore size filter (Millipore, USA).

E. Method validation

The analytical method was validated on specificity, precision, accuracy, linearity, range, and limits of detection and quantification.

F. Statistical calculations

Standard regression curve analysis was performed by using Micro-soft Office Excel 2007 software (Microsoft, USA), without forcing through zero. Means and standard deviations were calculated by using SPSS software version 9.5 (SPSS, Cary, NC, USA).

Results and Discussions

A. Specificity of the developed method

The specificity of this method was determined by analysis of the blank, placebo, standard and sample solution chromatograms (Figures 2-5). Good separation between luteolin-7-glucoside and matrix was achieved, with the retention times, 14.075 min by comparing chromatograms of blank, placebo, standard and sample, there was no interference observed from the peaks of the blank and placebo. It showed that the method is high specificity.

B. Linearity and range of the developed method

For linearity studied, five solutions in the ranges of 25-200 µg/ml for luteolin-7-glucoside were analyzed. Each concentration was made and analyzed in triplicate. The peak areas obtained from each concentration of the analytes were used to build a linear regression equation as well as determined the value of correlation coefficient (Table 1). Good linearity was observed over the above - mentioned range with linear regression equation Y= 19650x+ 134147 (x is concentration of analytes in µg/ml and Y is peak area). The values of correlation coefficient were 0.9995.

C. Accuracy of the developed method

This study was performed by adding known amounts of Luteolin-7-glucoside to the placebo samples. Three level of solutions were made and having concentrations at 50, 100, 150 µg/ml. The recovery range for luteolin-7-glucoside was 91.58 to 96.85 % (limit 80 to 110%).[4,5] The relative standard deviation ranged from 0.41 to 1.72 %.

D. Precision of the developed method

Repeatability was studied by calculating the relative standard deviation (RSD) from six determinations of the 100% concentration of sample. The studied was performed on the same day and under same experimental conditions. The concentrations of luteolin-7-glucoside determinations in the sample solution with the relative standard deviation were calculated (Table 3). The RSD value obtained for Luteolin-7-glucoside was 0.46. (limit less than 5.3%).[4,5] The result showed that the developed method was precise.

E. Sensitivity of the developed method

LOD were calculated by using the following equations. LOD = 3.3 x SD/S and LOQ = 10 x SD/S, where SD = the standard deviation of the response, S = Slope of the calibration curve. The LOD values were 5.76 µg/ml and the LOQ values were 19.19 µg/ml of luteolin-7-glucoside, respectively. Method validation following ICH guidelines indicated that the developed method had high sensitivity.

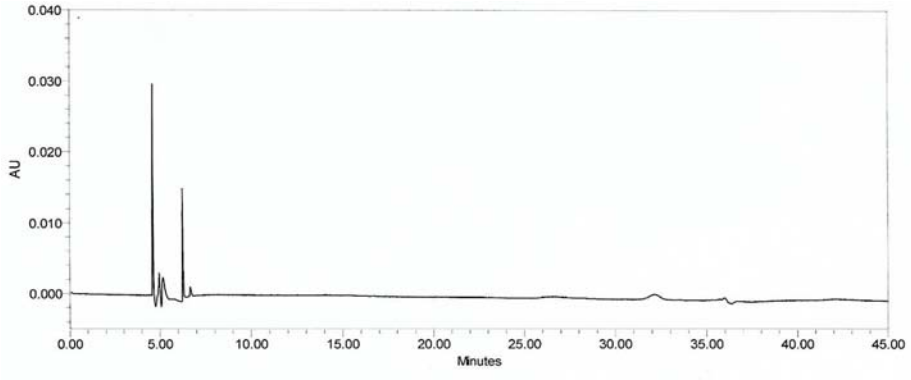

Figure 2. HPLC Chromatogram of blank solutions

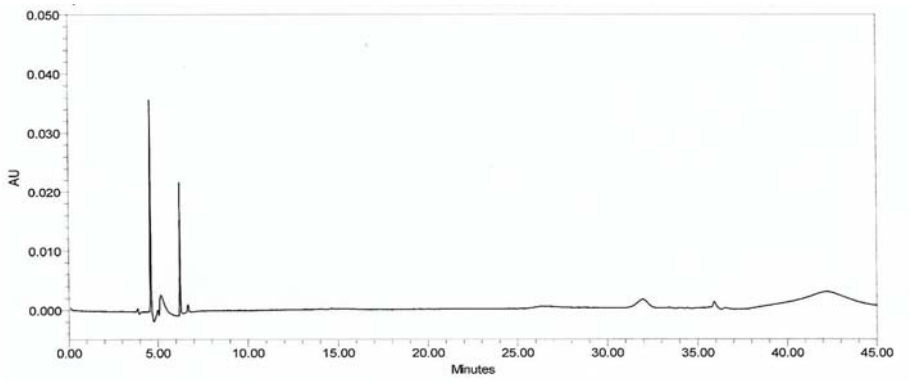

Figure 3. HPLC Chromatogram of Placebo Solutions

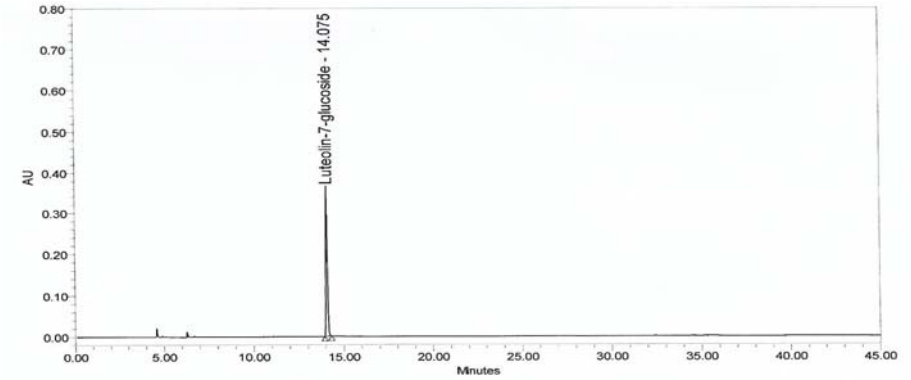

Figure 4. HPLC Chromatogram of luteolin-7-glucoside (standard solutions)

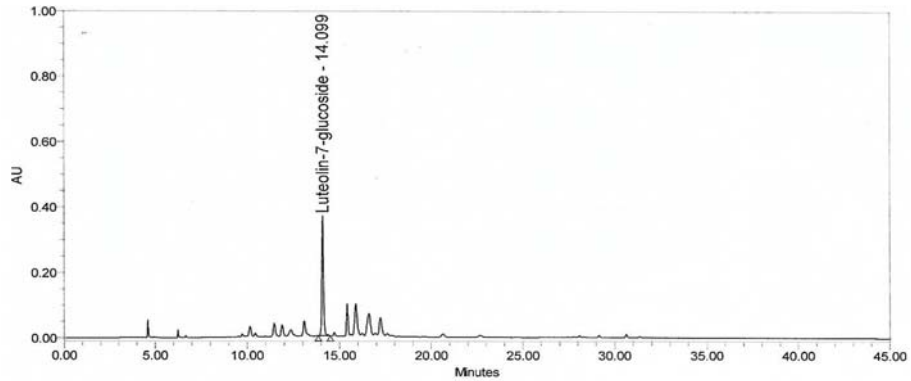

Figure 5. HPLC Chromatogram of sample solutions

Table 1. Linearity and range for luteolin-7-glucoside by HPLC

Sample number	Luteolin-7-glucoside	
	Concentration (µg/mL)	Peak area
1	25	646,249
2	50	1,115,304
3	100	2,088,288
4	150	3,006,030
5	200	4,102,727

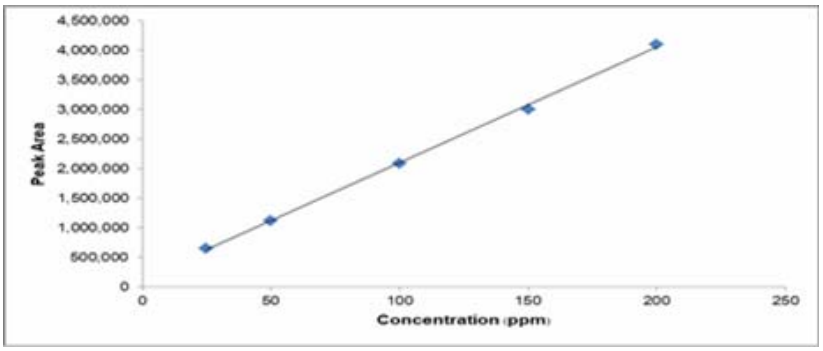

Figure 6. Calibration Curve of Luteolin-7-glucoside by HPLC

Table 2. Accuracy data of luteolin-7-glucoside by HPLC

Compounds	Amount (µg /mL)	% Recovery	% RSD
Luteolin-7-glucoside	50	96.84	0.41
	100	95.38	1.72
	150	91.58	1.24

Table 3.Precision studies of luteolin-7-glucoside by HPLC

N	% W/W
	Luteolin-7-glucoside
1	0.0057
2	0.0057
3	0.0057
4	0.0057
5	0.0055
6	0.0057
% RSD	0.46

Conclusion

Using this method, luteolin-7-glucoside could be determined and the validity of the method was also verified. The proposed analytical method for estimation of luteolin-7-glucoside in the Chrysanthemum flower capsules is accurate, precise, linear, robust, reproducible and within the range.

Acknowledgements

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