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Evaluation of Chlorogenic Acid in *Flos lonicerae* by HPLC Method

LIAO WEIPING†‡ and RUI YUKUI*†
College of Resources and Environmental Sciences,
China Agricultural University, Beijing 100193, P.R. China
Fax: (86)(10)62731016; Tel: (86)(10)62733617; E-mail: ruiyukui@163.com

High-performance liquid chromatography has been evaluated for the determination of chlorogenic acid in *Flos lonicerae*, primarily by the separation of chlorogenic acid. The samples were extracted at reflux by methanol and the content of chlorogenic acid was analyzed by HPLC. A diamonsil C_{18} column (4.6 mm × 150 mm, 5 μm) was used as the analytical column. The mobile phase was methanol, water and phosphoric acid (20:80:0.1). The detection wavelength was set at 327 nm to determine the content of chlorogenic acid. The chlorogenic acid showed linearity over the range of 8.8-88 μg/mL (r = 0.9999). Its average recovery rate was 98.1 % and RSD was 1.85 % (n = 5). The accuracy of the method was 0.82 % (n = 5).

Key Words: HPLC, *Flos lonicerae*, Chlorogenic acid.

INTRODUCTION

Traditional Chinese medicines (TCM) played an important role in the health of the Chinese people for thousands of years. Today, traditional Chinese medicines have been developed for a variety of medicinal uses to prevent disease. Natural products are the most important resources of anticancer agents, where over 60 % of the approved and pre-new drug application candidates are either natural products or synthetic molecules based upon the natural product molecular skeletons from TCM [1]. *Flos lonicerae* (Jinyinhua in Chinese), the dried buds of several species of the genus Lonicera (Caprifoliaceae), is a commonly used traditional Chinese medicine herb. It has been used for centuries in TCM practice for the treatment of many diseases[2,3].

Chlorogenic acid (3-O-ceffeoyl-d-quinic acid) is one of the most effective compounds found in the traditional Chinese medicinal herb *Flos lonicerae*. It can serve as antioxidant, antitumor, antimutagenic and anticarcinogenic agents[4,5]. To secure the quality of Chinese traditional medicine, it is important to determine the content

†Yuanmingyuan Xilu, No. 2, Haidian District, Beijing 100193, P.R. China.
‡Topsun Science & Technology Stock Co., Ltd Xi'an Pharmaceutical Plant, Xi'an City, Shsnxin Province 710075, P.R. China.

of chlorogenic acid. Many methods have been patented to detect chlorogenic acid in Chinese traditional patent medicine[6-8], but no united method for the detection of chlorogenic acid in *Flos lonicerae* has been reported. This study is to establish the method of detecting chlorogenic acid in *Flos lonicerae*.

EXPERIMENTAL

The high-performance liquid chromatography system consisted of a waters 600 liquid pump, a waters 2487 UV tunable absorbance detector and Empower data processing system. A Sartorius BP211D electronic balance and KQ-250DE ultrasonic cleaner were also used. HPLC-grade acetonitrile was used as received from TEDIA (Fairfield, USA). Double distilled water and all other chemicals were analytical grade (Xi'an Chemical Plant, Shanxi province China). A standard sample of chlorogenic acid was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, batch number 110753-200212).

Procedure for the extraction of chlorogenic acid from *Flos Lonicerae*: To extract chlorogenic acid, the *Flos Lonicerae* was decocted in 8 times the volume of boiling water for 2 h, then decocted in 6 times the volume of boiling water for 2 h. The extracted solutions were mixed and filtered. The solution was then adjusted to pH 10-12 with $Ca(OH)_2$. The resulting precipitate was collected by filtration, dissolved with water and adjusted to pH 6-7 with H_2SO_4, filtered and dried at 60 °C. This was then powdered and sieved through a 100 mesh. The extracted chlorogenic acid was weighed and dissolved in 50 % methanol and stored for measurement. The control solution of chlorogenic acid was prepared in the same manner.

Chromatographic conditions: A Diamonsil C_{18} column (250 mm × 4.6 mm × 5 μm) was used for the separation of chlorogenic acid from samples. The column was maintained at room temperature throughout the analytic process and the eluant was monitored at 327 nm. The mobile phase was methanol-water-phosphate (20:80:0.1, v/v) at a flow rate of 1.0 mL/min. The number of theoretical plates calculated from the chlorogenic acid peak was no less than 2000.

RESULTS AND DISCUSSION

Interference experiment on blank: 10 μL of these solutions of chlorogenic acid were injected in HPLC to determine the concent of chlorogenic acid, the resulting chromatographic graph is shown in Fig. 1. The chromatographic graph showed that the components in the negative control do not disturb the detection of chlorogenic acid.

Standard curve: Different concentrations of chlorogenic acid solution were prepared from the mother liquor to give six solutions; 8.8, 22.0, 44.0, 66.0 and 88.0 μg/mL. The concentration of chlorogenic acid was detected using the standard method with an injection volume of 10 μL. The standard curve, was shown in Fig. 2.

$$Y = 44.8502X - 0.1242, r = 0.9999$$

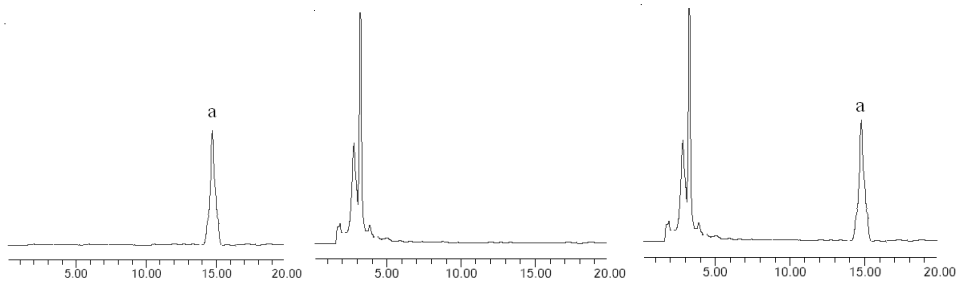

Fig. 1. HPLC chromatographic graph of chlorogenic acid (1) control of chlorogenic acid; (2) negative control of chlorogenic acid; (3) sample

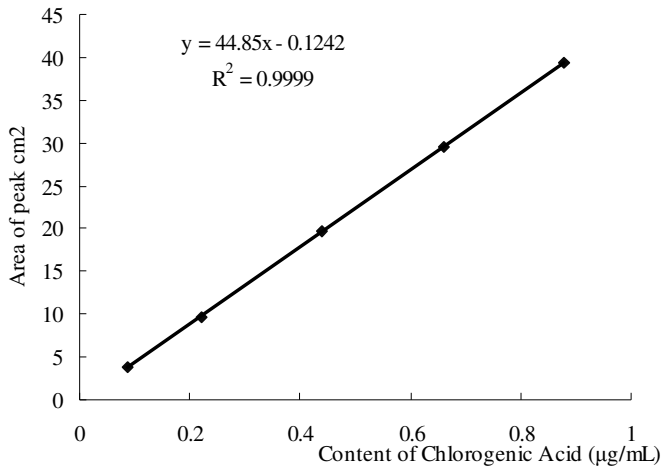

Fig. 2. Standard curve of chlorogenic acid

The results showed that the contentrtion of chlorogenic acid was in a good linearity with the area of peaks over the range 8.8-88 μg/mL (r = 0.9999).

Accuracy experiment: The control solution of chlorogenic acid (44.0 μg/mL, 10 μL) was injected five times at the same chromatographic conditions and the results are showed in Table-1. The RSD (0.82 %) showed that the method is precise.

TABLE-1
RESULTS OF ACCURACY EXPERIMENT

Replications	1	2	3	4	5
Area of peak (cm^2)	19.5890	19.4972	19.3296	19.2678	19.6321
RSD (%)			0.82		

Stability study: The extract from the same batch was repeatedly injected into HPLC at 2 h intervals over 8 h after the extraction. The results showed that the samples are stable over 8 h (Table-2).

TABLE-2
RESULTS OF STABILITY STUDY

Detecting time (h)	0	2	4	6	8
Area of peak (cm^2)	13.7021	13.6327	13.1872	13.4576	13.6279
RSD (%)			1.53		

Repeatability: Six sample solutions were made from the same batch of *Flos lonicerae* (batch number 001) and the content of chlorogenic acid and the RSD (%) was determined according to the above method. The results are showed in Table-3. The resulting RSD indicates the method is repeatable.

TABLE-3
RESULTS OF REPEATABILITY EXPERIMENT

Number	1	2	3	4	5	6
Area of peak (cm^2)	13.6987	13.5430	13.2712	13.7420	13.5897	13.6466
RSD (%)				1.24		

Recovery: 0.15 g Chlorogenic acid from the extraction (batch number 001) was accurately weighed into each of five conical flasks. A standard addition of 2.90 mg chlorogenic acid was made to each flask and the chlorogenic acid was measured using the the above procedure. The recovery (%) was calculated by the following formula. The results are shown in Table-4.

$$\text{Recovery}\,(\%) = \frac{\text{Detected value} - \text{content in sample}}{\text{Weight of standard sample}\,(\text{mg})} \times 100$$

The results showed that the recovery of this method varies from 96.1-100.2 %.

TABLE-4
RESULTS OF RECOVERY EXPERIMENT (mg)

No.	Content in sample	Added content	Detecting value	Recovery (%)
1	2.9001	2.9001	5.7770	99.2
2	2.8800	2.9002	5.7164	97.8
3	2.9102	2.9000	5.7290	97.2
4	2.8900	2.8994	5.7952	100.2
5	2.8701	2.8786	5.6364	96.1
Average		98.1		
RSD (%)		1.65		

Determination the content of three batches of *Flos lonicerae*: The contents of chlorogenic acid in three batches (Batch 1, 2 and 3) of *Flos lonicerae* were determined by the above method and the results are shown in Table-5.

The results suggested that these three batches of *Flos lonicerae* contain more chlorogenic acid than the pharmacopoeia standard (0.15 %).

TABLE-5
DETECTION RESULTS OF THREE BATCHES *Flos lonicerae*

Batch number	Content of chlorogenic acid (%)
1	0.171
2	0.160
3	0.158

The main aim of this study was to establish a HPLC method that is suitable for determination of chlorogenic acid in the Chinese traditional medicine *Flos lonicerae*. The data showed that the chlorogenic acid displayed good linearity over the range of 8.8-88 μg/mL (r = 0.9999), its average recovery was 98.1 % and RSD was 1.85 % (n = 5), the accuracy of the method RSD was 0.82 % (n = 5). To the best of our knowledge, this method meets the requirement of the present pharmacokinetic studies of chlorogenic acid in *Flos lonicerae*.

On comparing with the previous method[9], this method lowered the detection limit from 20 to 8.8 μg/mL, increased the relative coefficient (r^2) to 0.9999 from 0.9839 of method of flow injection with chemiluminescence detection[10] and increased the average recovery rate from 97.58[11] to 98.1 %, so the method presented here was simple, specific and accurate.

Conclusion

The method to evaluate chlorogenic acid in *Flos lonicerae* is as follows: the content of chlorogenic acid was analyzed by HPLC. A diamonsil C_{18} column (4.6 mm × 150 mm, 5 μm) was used as the analytical column. The samples were extracted by refluxing extraction of methanol, the mobile phase was methanol, water and phosphoric acid (20:80:0.1). The detection wavelength was set at 327 nm to determine the content of chlorogenic acid.

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