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SHORT COMMUNICATION

EVALUATION OF BAICALIN IN *SCUTELLARIA BAICALENSIS GEORGI* USING HPLC METHOD

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ABSTRACT. High-performance liquid chromatography (HPLC) has been evaluated for the determination of components in *Scutellaria baicalensis georgi*, primarily by the separation of baicalin. The samples were extracted at reflux by methanol, and the content of chlorogenic acid was analyzed by HPLC. A Diamonsil C18 column (4.6 mm x 150 mm, 5 μm) was used as the analytical column. The mobile phase was methanol, water and phosphoric acid (47:53:0.2). The detection wavelength was 280 nm to determine the content of baicalin. The baicalin showed linearity over the range of 0.12-1.2 μg. Its average recovery was 98.6% and RSD was 0.78%.

KEY WORDS: High performance liquid chromatography, *Scutellaria baicalensis georgi*, Baicalin

INTRODUCTION

Scutellaria is a perennial plant herbage and its root can be used for medicine to reduce fever and associated 'sweats', reduce inflammation, improve circulation, as well as be a fetal sedative. Recently, studies have been conducted on its active components, and have found that *Scutellaria* root extract showing anti-bacterial [1], anti-viral [2], anti-cancer, fever control, analgesic, anti-oxidation and free-radical scavenging as well as application in the treatment of heart diseases [3-5]. Gu's [6] showed that baicalin could induce apoptosis and inhibit the proliferation of prostate cancer cell DU145 in a dose and time-dependent manner.

The Chinese traditional medicine baikal skullcap root has been used to treat many diseases by anti-oxidation and radical scavenging mechanisms. Baicalin is the main component of baikal skullcap root, however, it does not exists in a free form but does as associated with metals in solution. The inhibition effects of baicalin and baicalin combined with each of six metal elements were examined by UV-Vis spectroscopy. The results indicated that all the six metal elements improve the antioxidant action of baicalin, but the effects and mechanisms were different from free baicalin [7].

In summary, baicalin is one of the most effective components of *Scutellaria.* To ensure the quality of Chinese traditional medicine, it is important to determine the content of baicalin. Many methods have been developed to detect baicalin [8-10] in Chinese traditional patent medicine, nevertheless, few methods for the detection of baicalin in *Scutellaria baicalensis georgi* has been reported till now. This study is to establish a simple, specific and accurate method of detecting baicalin in *Scutellaria baicalensis georgi.*

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EXPERIMENTAL

Instruments. HPLC 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 used.

Chemicals. HPLC-grade acetonitrile that was received from TEDIA (Fairfield, USA) was used. All the water was doubly distilled and all the other chemicals were analytical grade (Xi'an Chemical Plant, China).

Materials. A standard sample of baicalin was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, batch number 110715-200212).

Extraction of baicalin from Scutellaria. The *Scutellaria* was decocted in 10 times the volume of boiling water for 1 h, then decocted in eight times the volume of boiling water for 1 h. The extracted solutions were mixed and filtered. The solution was then adjusted to pH 2 using HCl. The resulting precipitate was collected by filtration, washed using ethanol, and then dried below 60 °C. This precipitate was then powdered and, sieved through a 100 mesh. The extracted baicilin was weighed and dissolved in 50% methanol and stored for measurement. The control solution of baicilin was prepared in a similar manner.

Chromatographic conditions. A Diamonsil C_{18} column (250 mm × 4.6 mm × 5 μm) was used for the separation of baicalin from samples. The column was maintained at room temperature throughout the analytic process and the eluant was monitored at 280 nm. The mobile phase was methanol-water-phosphate (47:53:0.2, v/v) at a flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

Interference experiment on blank. 10 μL of the solutions were injected into HPLC, using the chromatographic system described above, to determine the concentration of baicalin, the resulting chromatogram is shown in Figure 1. The chromatogram showed that the components in the negative control did not disturb the detection of baicalin.

Standard curve. Different concentrations of baicalin solution were prepared from the mother liquor to give six solutions; 0.06, 0.12, 0.3, 0.6, 0.9, and 1.2 μg/mL. The concentration of baicalin was detected using the standard method with an injection volume of 10 μL. The results showed that the content of baicalin was in a good linearity with the area of peaks over the range 0.12-1.2 μg (r = 0.9994).

Accuracy experiment. The control solution (60.0 μg/mL, 10 μL) was injected five times using the same chromatographic conditions, and the results are given in Table 1. The RSD showed that the method was precise.

Table 1. Precision assessment in different replications.

Replications	1	2	3	4	5
Area of peak	25.5069	25.5074	25.4872	24.8768	25.2873
RSD (%)	1.07				

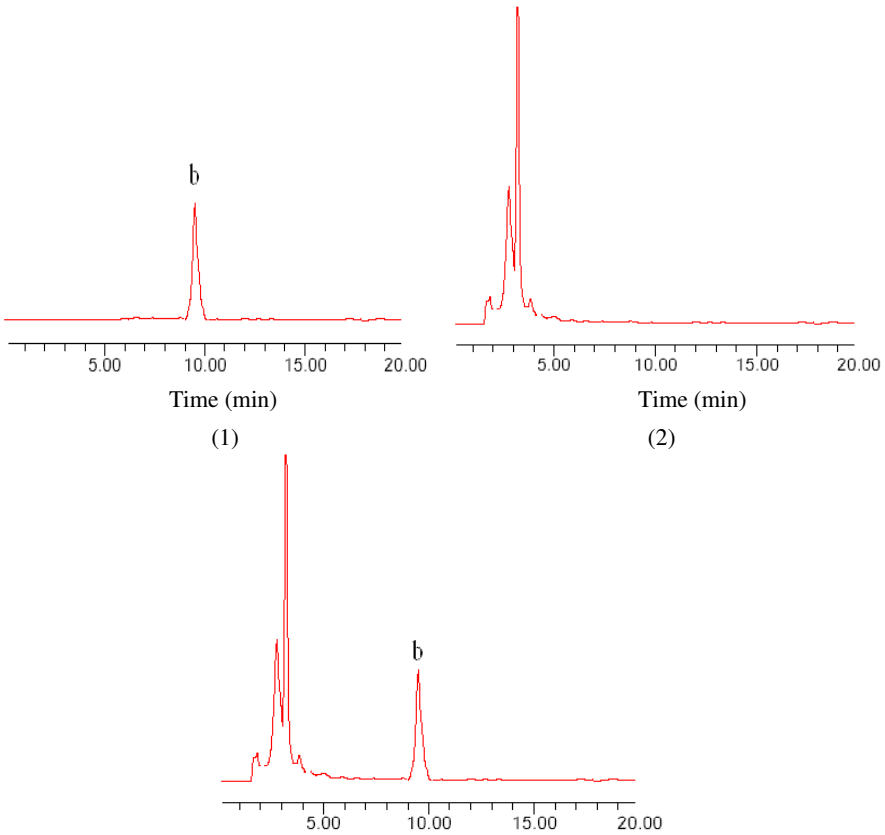

Figure 1. HPLC chromatogram of baicalin: (1) control of baicalin; (2) negative control of baicalin; (3) sample.

Stability study. The extract from batch number 001 was repeatedly injected into HPLC at 2 h intervals over 8 h after the extraction. The data showed that the samples were stable over 8 hours (Table 2).

Repeatability experiment. Six sample solutions were made from the same batch of *Scutellaria* (batch number 001), and the content of baicilin and calculating the RSD (%) was determined according to the above method. The data are given in Table 3. The resulting RSD indicates the method is repeatable.

Table 2. The assessment of stability at different time.

Detecting time	0	2	4	6	8
Area of peak	26.4388	26.4120	25.8731	25.5798	26.3929
RSD (%)	1.50				

Table 3. Repeatability experiment.

Number	1	2	3	4	5	6
Area of peak	26.4297	26.4368	26.0712	25.6731	25.5989	26.3412
RSD (%)	1.45					

Recovery. 0.05 g baicilin from the extraction (batch number 001) was accurately weighed into each of five conical flasks. A standard addition of 3.0 mg baicilin was made to each flask, and the baicilin was measured using the standard procedure. The recovery (%) results are given in Table 4. The results should that the recovery of this method is high.

Table 4. Results of recovery experiment (mg).

No.	Content in sample	Added content	Detected value	Recovery (%)
1	3.0621	3.0012	6.0241	98.7
2	3.0782	3.0052	6.0564	98.5
3	3.0053	3.0121	5.9511	97.8
4	3.0020	3.0090	6.0050	99.8
5	3.0281	3.0111	5.9820	98.1
Average	98.6			
RSD (%)	0.78			

Determination the content of three batches scutellaria. The contents of three batches (Batch 1, Batch 2 and Batch 3) of *Scutellaria* were determined by the standard method and the results are shown in Table 5.

Table 5. Detection results of three batches of *Scutellaria.*

Batch number	Content of baicilin (%)
1	9.8
2	9.3
3	9.1

The results showed that these three batches of *Scutellaria* contained more baicilin than the Pharmacopoeia standard (9.0%).

The main aim of this study was to establish a HPLC method that is suitable for determination of baicalin in the Chinese traditional medicine *Scutellaria*. The results showed that the baicalin displayed a good linearity over the range of 0.12-1.2 μg ($r = 0.9994$), its average recovery was 98.6% and RSD was 0.78% ($n = 5$).

Compared with the earlier methods, this method lowered the detection limit from 0.2088 μg/mL [9] to 0.12 μg/mL, and increased the average recovery rate from 96.3% to 98.6% [11], so the method presented here is simple, specific and accurate.

CONCLUSIONS

A method using HPLC to evaluate baicalin content in *Scutellaria* is presented. A content of baicalin was analyzed by HPLC. A Diamonsil C18 column (4.6 mm x 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 (47:53:0.2). The detection wavelength was at 280 nm to determine the content of baicalin. The method presented here is fairly simple,

specific and accurate. High accuracy and stability of the results showed that this method is trustworthy and valuable for measurement of the baicalin content in *Scutellaria.*

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