Determination of Effective Components of Scutellaria indica from Different Habitats

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Abstract: A HPLC method for the determination of three effective components in Scutellaria indica from different habitats was established. Analysis was performed on Waters Spherisorb ODS2 (4.6 mm × 250 mm, 5 μm) with gradient mobile phase of 0.5% glacial acetic acid aqueous solution- acetonitrile. The flow rate was 1 ml/min and the detection wavelength was set at 275 nm. The results showed that the chromatographic elution curves with good resolution and reproducibility were obtained. By comparing with the reference materials, three effective components were determined, namely, scutellarin, scutellarin and apigenin. The method is sensitive, accurate and repeatable, and can be used for the determination of three components in Scutellaria indica. The results also showed that there were significant differences in the chemical composition of Scutellaria indica from different areas. The quality differences of Scutellaria indica from different areas should be considered in the selection of Scutellaria indica.

1. Introduction
Scutellaria indica is a dry whole herb of Scutellaria Linn. It has the functions of hemostasis, detumescence, clearing away heat, detoxification, promoting blood circulation and relieving pain[1]. Although it is not included in Chinese Pharmacopoeia, it is commonly used in clinic as a local characteristic medicine. The chemical components of Scutellaria indica are mainly flavonoids. At present, there are more than 70 flavonoids isolated or detected from Scutellaria indica, but there are not many reports on the content determination. And that there are differences in the content of the same compound in Scutellaria indica according to different literature reports, which may be caused by different origins of Scutellaria indica, different extraction methods and detection methods of samples and other factors.

In this paper, 10 batches of Scutellaria indica from different areas were studied by HPLC[2-10]. The contents of scutellarin, scutellarin and apigenin in Scutellaria indica were determined by external standard method, in order to find out the differences of the contents of effective components in Scutellaria indica from different areas, and provide reference for the quality control and further development and utilization of Scutellaria indica.

2. Materials and materials

2.1 Instrument
Agilent 1200 HPLC, Agilent Technology (China) Co., Ltd; Electronic balance (EL104), Mettler Toledo Instruments Co., Ltd; Chinese medicine pulverizer (JP-500C), Zhejiang Yongkang Jiupin industry and Trade Co., Ltd;
2.2 Reagent
Control substance: scutellarin (batch No.: BZP0017) was purchased from Hefei Bomei Technology Co., Ltd.; scutellarin (batch No.: 180216) and apigenin (batch No.: 180424) were purchased from Shanghai Ronghe Pharmaceutical Technology Development Co., Ltd., with purity > 98%.

Reagents: acetonitrile (chromatographically pure), methanol (chromatographically pure), glacial acetic acid (analytically pure), all purchased from Tianjin comio Chemical Reagent Co., Ltd;
10 batches of Scutellaria indica from Different Habitats is identified as Scutellaria indica dried whole herb by Professor Wang Jiguang of Xuzhou pharmaceutical branch of Jiangsu United vocational and technical college.

3. Methods and results

3.1 Chromatographic conditions
Chromatographic column: Waters Spherisorb ODS2(4.6 mm×250 mm,5 um); mobile phase: 0. 5% glacial acetic acid(A)- acetonitrile(B); gradient elution model(0~20min, 90~85%A; 20~40min, 80~65%A, 40~60min, 65~35%A; 60~85min, 35~30%A); the wavelength: 275 nm; the flow rate :1.0mL/min, the temperature: 30℃.The HPLC chromatogram is shown in Figure 1.

3.2 Preparation of reference solution

| Number | I   | II  | III | IV  | IV  | VI  |
|--------|-----|-----|-----|-----|-----|-----|
| scutellarin | 14  | 28  | 56  | 84  | 112 | 140 |
| baicalin  | 15  | 30  | 60  | 90  | 120 | 150 |
| apigenin  | 8   | 16  | 32  | 48  | 64  | 80  |

Table 2 Regression equations, correlation coefficients and detection limits

| Reference solution | Regression equations | Correlation coefficients | Linearity range |
|--------------------|----------------------|--------------------------|-----------------|
| scutellarin        | Y=5.648X+4.394       | 0.9998                   | 7~70            |
| baicalin           | Y=3.028X+8.153       | 0.9996                   | 3~30            |
| apigenin           | Y=20.69X+7.665       | 0.9993                   | 8~80            |

Accurately weigh appropriate amount of scutellarin reference substance, reference baicalin, reference apigenin, obtain series of solutions as table 1.

3.3 Preparation of test solution
Take all batches of dried Scutellaria indica herbs and crush them, over 80 days. Accurately weigh 0.5g sample, place it in a conical flask with a stopper, add 60ml and 30ml of 70% methanol solution in turn, respectively, extract it with ultrasonic assistance for 45min, cool it and filter it, transfer it into a 10ml volumetric flask, fix the volume with methanol.

3.4 Linear range investigation
Take the mixed control solution I, II, III, IV, V and VI under "2.2" and record the peak area of response signal. The standard curves of scutellarin, baicalin and apigenin are drawn with concentration as abscissa and peak area as ordinate, and the linear equation and correlation coefficient are obtained. The standard curves are shown in Fig. 2, 3 and 4. See Table 2 for regression equation, linear range. The results show that there is a good linear relationship between the concentration of the three solutions and the peak area in the linear range.
3.5 Precision test
Take the same batch of Scutellaria indica (S05), prepare these Scutellaria indica (S05) according to the experimental method in "2.3", and then set up the instrument chromatography according to the method in "2.1" Conditions, and continuously Sample 5 times. The RSD of scutellarin, scutellarin and apigenin were 1.02%, 0.45% and 1.32%, respectively, which were less than 3%, indicating that the precision of the instrument was good.

![HPLC chromatograms of sample solution(S05) and reference solution](image)

Fig.1 HPLC chromatograms of sample solution(S05) and reference solution

![The standard curves of scutellarin](image)

Fig.2 The standard curves of scutellarin

![The standard curves of baicalin](image)

Fig.3 The standard curves of baicalin

![The standard curves of apigenin](image)

Fig.4 The standard curves of apigenin

3.6 Stability test
Take the same batch of Scutellaria indica (S05) and prepare one sample solution according to the experimental method under "2.3". Set the instrument chromatographic conditions according to the method under "2.1" at 0, 2, 4, 8, 12, and 24 hours after preparation respectively. The RSD of the content of scutellarin, scutellarin and apigenin is 1.76%, 1.98% and 2.03%, respectively, which are less than 3%, indicating that the sample is stable within 24 hours.

3.7 Recovery test
Take the same medicine S05 with known contents of scutellarin, scutellarin and apigenin, 9 parts, each
of which is about 0.5g. The sample solution was divided into three groups. The first three sample solutions were respectively added with 0.50ml of scutellarin, baicalin and apigenin stock solution by pipette gun, and the second three sample solutions were respectively added with 1.00mL of scutellarin, baicalin. Add 1.50ml of scutellarin, baicalin and apigenin respectively into the third group of three sample solutions with a pipette gun, and use methanol to fix the volume to 25ml volumetric flask, as the test solution. According to the method under "2.1", inject samples, and calculate the recovery rate and RSD of scutellarin, baicalin, apigenin respectively. The results showed that RSD was less than 3%, which indicated that the method was accurate and reliable.

3.8 Content determination results
Take 10 batches of Scutellaria indica samples, prepare the sample solution according to the method under "2.2", inject the sample for determination according to the chromatographic conditions under "2.3", and calculate the content of 3 components in Scutellaria indica. See Table 3 for the results.

4. Discussion
In this study, the mobile phase systems of water acetonitrile, water methanol, glacial acetic acid aqueous solution acetonitrile and glacial acetic acid aqueous solution ethanol were investigated respectively. After repeated optimization and comparison, 0.5% glacial acetic acid aqueous solution acetonitrile was used as the mobile phase for gradient elution, and the separation effect was the best. The results show that the three components have a large absorption at 275nm, and 275nm is selected as the detection wavelength. In this study, six columns (Agilent Eclipse XDB-C18, Waters Spherisorb ODS2, Thermo Acclaim-C18, Kromasil Enternity 5-C18, Symmetry shield RP18, Lichrospher NH2) were used respectively, and their spectrograms were compared. It was found that the two columns, Symmetry shield RP18 and Lichrospher NH2, had poor peak shape, few peaks and poor separation; the four columns (Agilent Eclipse XDB-C18, Waters Spherisorb ODS2, Acclaim-C18, Kromasil Enternity) had complete peaks and good separation effect. Finally, the Waters Spherisorb ODS2 column was selected to analyze the samples.

| Number | Scutellarin(mg/g) | Baicalin(mg/g) | Apigenin(mg/g) |
|--------|------------------|---------------|----------------|
| S01    | 0.672            | 0.612         | 0.374          |
| S02    | 1.471            | 1.159         | 0.689          |
| S03    | 0.879            | 0.413         | 0.412          |
| S04    | 2.104            | 0.668         | 0.812          |
| S05    | 1.457            | 1.063         | 0.686          |
| S06    | 1.886            | 0.839         | 0.823          |
| S07    | 1.393            | 0.988         | 0.667          |
| S08    | 0.861            | 0.631         | 0.419          |
| S09    | 0.951            | 0.730         | 0.461          |
| S10    | 1.939            | 1.524         | 1.013          |

5. Conclusion
In this study, the extraction effects of different solvents (water, ethanol, methanol, 50% methanol and 70% methanol), extraction methods (ultrasonic extraction and reflux extraction), extraction time (0.5, 1.0, 1.5, 2.0 h) and extraction dosage (0.1, 0.5, 1.0, 1.5, 2.0 g) were compared. The results showed that when water was used as extraction solvent, the number of peaks was small and the separation was incomplete. When ethanol was used as extraction solvent, some peaks disappeared, and the HPLC patterns of different concentrations of methanol solvent extracts were basically the same. It was found that 70% methanol solution was used as extraction solvent, the peak shape of the chromatogram of Scutellaria indica extract was the best; the extraction efficiency of ultrasound is higher than that of reflux extraction, and compared with reflux extraction, ultrasonic extraction is simple, cost-effective and time-saving. As for extraction time, with the increase of extraction time, the extraction efficiency is
significantly improved, but it will not change after 1.5 hours; the difference of the spectrum obtained by different extraction amount is not big, but when the amount is increased, the concentration is also increased, so it is difficult to filter. Therefore, in order to facilitate the operation, the extraction amount is selected as 0.5g to reduce the error.

In this study, a HPLC method for the determination of three flavonoids in Scutellaria indica was established. Under this experimental condition, the content of scutellarin, scutellarin and apigenin in Scutellaria indica has good precision, repeatability, stability and high recovery, it has a good linear relationship in the corresponding linear range. This method can be used for the determination of three components in Scutellaria indica, and it also provides an important reference for the quality control of Scutellaria indica.

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