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Determination of Scutellarin in *Scutellaria barbata* Extract by Liquid Chromatography–Electrochemical Detection

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ABSTRACT

A rapid and simple method was developed for the determination of scutellarin in *Scutellaria barbata* plant extracts by reversed-phase liquid chromatography with an electrochemical detector (ECD). The potential electrode voltage was +0.7 V for the ECD. The mobile phase consisted of methanol:water:glacial acetic acid (30:68:2, v/v/v) (pH 2.7). The linear calibration ranged from 2.3 to 150.0 $\mu\text{g mL}^{-1}$ for scutellarin. The detection limit was 0.2 $\mu\text{g mL}^{-1}$ given a signal-to-noise ratio (S/N) of 3:1 on the second detection fullscale (500 nA). The six different extracts of *S. barbata* were determined using different extraction methods and extraction solvents. The contents of scutellarin in the extracts were determined within 25 min.

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Key Words: Scutellarin; Liquid chromatography; Electrochemical detection.

INTRODUCTION

Scutellarin belongs to one of the glycosides metabolized from flavones. These compounds are important phytochemicals, and some of them are biologically active. Scutellarin can prevent agglutination of blood platelets and can pass through the blood–brain barrier.^[1,2] As a natural drug, scutellarin is being developed for the treatment of cerebrovascular diseases such as hemiplegia. Scutellarin can be derived from several species of plants such as *Scutellaria barbata*, *Erigeron breviscapus*, etc. *Scutellaria barbata* is a perennial herb, which is natively distributed throughout Southern China. This herb is named as Ban-Zhi-Lian in traditional Chinese medicine. It is used as an anti-inflammatory agent, an antitumor agent, and a diuretic.^[3]

Currently, there have been some reports on the quantitation of scutellarin including thin layer chromatography (TLC),^[4] liquid chromatography–mass spectrometry (LC–MS),^[5] and so on. Scutellarin contains hydroxyl groups of phenol, which is electroactive and can be determined by an electrochemical detector (ECD). High-performance liquid chromatography coupled with an electrochemical detector (HPLC/ECD) is widely used, because it offers excellent selectivity and sensitivity, especially for complex samples.

In this paper, we established a simple method for the quantitation of scutellarin in *S. barbata* extract by reversed-phase HPLC/ECD. The method is sensitive and reproducible. We used six different procedures for the extraction of *S. barbata* and compared the results. Using this method, the quantitation of scutellarin in other plant extracts can be accomplished without a complicated clean-up procedure. To our knowledge, this is the first report for the quantitation of scutellarin using ECD.

EXPERIMENTAL

Chemicals

Scutellarin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other chemicals were purchased from Shanghai or Tianjing Chemical Reagent Corp. (China) and are of analytical-reagent grade. De-ionized water was used.

Scutellaria barbata was collected from Yunnan Province, China.





Apparatus and Chromatographic Conditions

An HPLC system (Agilent Technologies, USA) with an Agilent binary pump (Model 1100), an online degasser, and a Hewlett-Packard electrochemical detector (Model 1049A) was used in the present study. The analytical column (150 mm \times 4.6 mm I.D.), packed with silica gel C₁₈ of 5 μ m particle size (Chromatorex, Fuji Silysia Chemical LTD., Japan) was used. The chromatographic conditions were set as follows: the voltage of the potential electrode: 0.7 V on the second sensitive scale (0–500 nA); the mobile phase consisted of methanol : water : glacial acetic acid (30 : 68 : 2, v/v/v) (pH 2.7); flow-rate 1.0 mL min⁻¹. The mobile phase was filtered through a 0.45 μ m filter prior to use.

Sample Preparation

Six different procedures of extraction were investigated. Dry *S. barbata* was pulverized to fine powder. The first procedure was: 1.0 g of powder was put in a 100-mL volumetric flask with 90 mL of methanol. The flask containing the mixture was put in an ultrasonic washer for 30 min. Methanol was then added to the graduation on the flask after cooling to room temperature. The mixed solution was centrifuged at 4000 rpm for 10 min. The supernatant liquid was filtered through a screen with a pore size of 0.45 μ m. Twenty microliter of the filtrate was directly injected into the chromatographic column.

In the second procedure 1.0 g of powder was put in a boiling flask with 90 mL of methanol, then refluxed for 4 hr. The solution was then poured into a 100-mL volumetric flask after cooling and methanol was added to the graduation on the flask, then the following procedure was the same as the corresponding part of the first procedure.

In the third and the fourth procedures, water was used as the solvent instead of methanol, and the other conditions were the same as that of the first and the second procedure.

In the fifth and the sixth procedures, ethanol was used as the solvent instead of methanol, and the other conditions were the same as that of the first and the second procedure.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

We investigated the response of scutellarin at different potentials (Fig. 1). Figure 1 shows that the peak area of scutellarin changes with the oxidizing



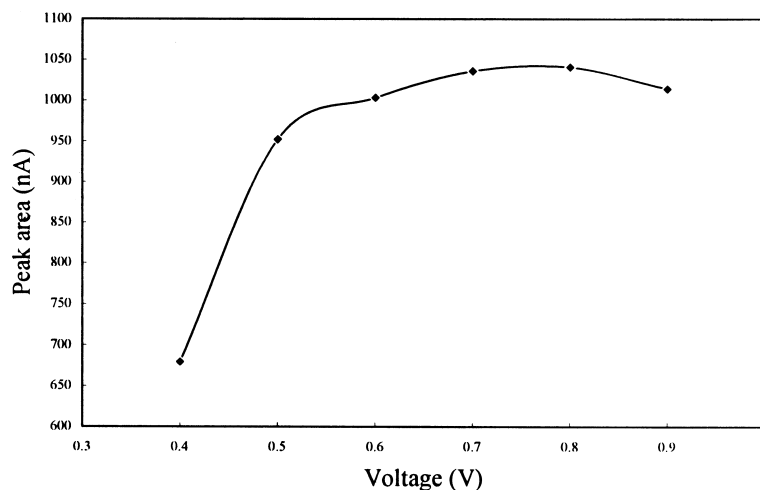


Figure 1. Voltammogram of scutellarin.

potential electrode voltage from 0.4 V to 0.9 V on the second sensitive scale (0–500 nA) when methanol:water:glacial acetic acid (30:68:2, v/v/v) (pH 2.7) are used as the mobile phase. From the figure, the response of scutellarin increases rapidly when the electrode is beyond 0.4 V, but the baseline noise is increased from 17.5 nA to 170 nA, simultaneously. The maximum response of scutellarin is reached at 0.8 V. Considering the high baseline noise using the high potential voltage, 0.7 V was selected as the potential electrode voltage for the ECD in this paper.

The percentage of methanol in the mobile phase was optimized at the mobile phase containing 2% of glacial acetic acid. In Fig. 2, the capacity factor of scutellarin is plotted vs. the percentage of methanol. Capacity factor (k') was calculated from $k' = (t_R - t_0)/t_0$; where t_R is the retention time; t_0 is the dead time, and t_0 is determined by the retention time of methanol in methanol:water:glacial acetic acid (30:68:2, v/v/v) (pH 2.7) used as the mobile phase. When the percentage of methanol increased from 20 to 40%, the capacity factor of scutellarin decreased obviously, which implies that the capacity factor of scutellarin is sensitive to the percentage of methanol in the mobile phase. The higher concentration of methanol led to more fused peaks for the plant extract on the chromatogram, so the concentration of methanol was selected at 30%. Hence, the optimum mobile phase containing methanol:water:glacial acetic acid (30:68:2, v/v/v) (pH 2.7) was used in the paper.



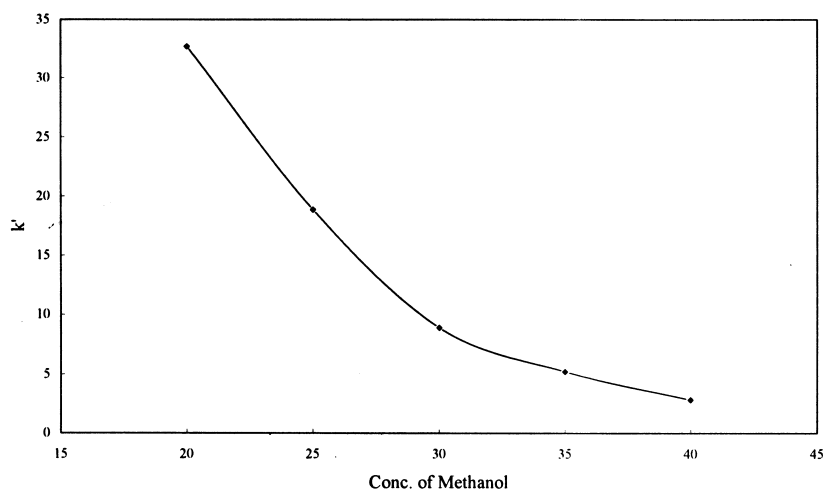


Figure 2. Effect of concentration of methanol in mobile phase on capacity factor of scutellarin.

Linearity and Sensitivity

Standard solutions were prepared in methanol at concentrations of 2.3–150.0 $\mu\text{g mL}^{-1}$. Calibration curve was obtained by plotting peak area vs. concentration of scutellarin. The regression equation and its correlation coefficient were calculated as follows:

$$A = 72.993 C - 149.53 (n = 7; r = 0.9998)$$

The detection limit corresponded to the concentration of 0.2 $\mu\text{g mL}^{-1}$ given a signal-to-noise ratio (S/N) of 3 : 1 on the second detection fullscale (500 nA).

Precision and Recovery

The precision and recovery of scutellarin are summarized in Table 1.

Quantitation of the Plant Extract

We determined six different extracts of *S. barbata* using different extraction methods mentioned above. The representative chromatograms are



Table 1. The results of within-day precision, day-to-day precision, and recovery (% , $n = 5$).

Con. ($\mu\text{g mL}^{-1}$)	Within-day precision	Day-to-day precision	Recovery
2.3	1.61	2.66	94.9
37.5	1.93	3.34	101.7
150.0	0.67	1.88	99.0

shown in Fig. 3. Contents of scutellarin in *S. barbata* calculated according to peak areas (see Table 2).

Using ethanol as the extraction solvent, scutellarin is extracted less than that using water and methanol, which indicates that ethanol is not suitable for the extraction of scutellarin. The concentrations of scutellarin using methanol

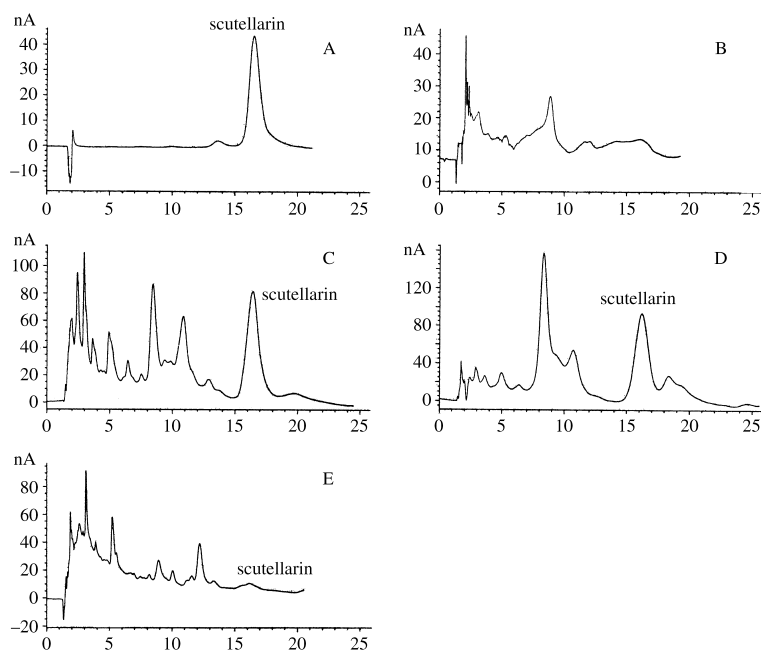


Figure 3. Representative chromatograms of scutellarin. A, standard scutellarin; B, *S. barbata* extract using ethanol as extraction solvent under reflux; C, *S. barbata* extract using water as extraction solvent under reflux; D, *S. barbata* extract using methanol as extraction solvent under reflux; E, *S. barbata* extract using water as extraction solvent under ultrasonic extraction.

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Table 2. Contents of scutellarin in *S. barbata* under different extraction conditions (mg g^{-1}).

Extraction solvent	Extraction method	
	Ultrasonic	Reflux
Water	0.62	7.43
Methanol	4.58	5.47
Ethanol	—	—

as the extraction solvent are higher either by ultrasonic extraction or by reflux. Though the concentration of scutellarin in water is very low with ultrasonic extraction, it is still the highest under the reflux condition. Comparing the six different extraction procedures, water is the best extraction solvent for scutellarin under the reflux condition. It is consistent with the opinion that decocting *S. barbata* herbs is helpful to medical absorption in the traditional Chinese medical culture.

Electrochemical detection can be used to determine trace electroactive compounds in plant sources because of its high selectivity and sensitivity. In this paper, the method for the determination of scutellarin in *S. barbata* extract is easy and very sensitive using ECD without complicated sample pretreatment. Currently, this method is being used routinely in our laboratory to quantify the concentrations of scutellarin in *S. barbata* extract for quality control of the traditional Chinese medicine.

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