Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):595-599

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Retrieving scutellarin from the acid precipitation waste liquid

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ABSTRACT

This research established an easy method to retrieve scutellarin from the acid precipitation waste liquid. The waste liquid in scutellarin production process was collected and concentrated. Then, it was added with one to five times volume of distilled water and the coarse-grain of scutellarin was collected as the sediment. Finally, the scutellarin with high purity (90.1-90.2%) was retrieved by recrystallization with absolute ethyl alcohol. The content of scutellarin in every step was determined by HPLC-UV method. This is a handy method to retrieve scutellarin from the acid precipitation waste liquid and it can increase the availability of crude material.

Key words: scutellarin; wastewater; retrieve

INTRODUCTION

Erigeron breviscapus, named as Dengzhangxixin in Chinese, is used to treat hemiplegia, chest pain, rheumatism, headache and toothache [1]. The1980's, Zhang Renwei, came from Yunnan Institute of Materia Medica, first obtained scutellarin (5,6,4′- three flavonol -7-O- three hydroxy flavone glucuronide) from it, and named the mixture which contains a large amount of scutellarin and a small amount of pigment as breviscapine [2]. The breviscapine tablet and injection have been developed based on the research. At present, the output value of *E. breviscapus* is more than billions of RMB every year. Now, *E. breviscapus* has been listed as the focus herbal medicine in Yunnan Province,, a large scale of planting and processing is under way.

Ordinarily, the breviscapine is separated and purified from *E. breviscapus* extract by acid precipitation and recrystallization [3]. Another method is enriching the total flavonoids by macroporous resin, acidifying the eluent to obtain coarse-grain of breviscapine, and then the high purity scutellarin is collected by recrystallization [4]. In order to prepare scutellarin for the Erigeron phenol, Sun et al. also adjusted pH value at 1-2 to subside the coarse-crystal of scutellarin, and further purified by recrystallization [5]. Zhang Renwei applied a patent, a method for preparing breviscapine with macroporous resin, which also contains the precipitation process by regulating pH value at 1-2 [6]. In all the above mentioned methods, the acid precipitation process has been adopted in production of breviscapine or scutellarin, which results in a large amount of acid waste liquid.

The pH value of such acid waste liquid is 1-2. In order to achieve emission standard, it requires lots of alkali to neutralize. At the same time, there is a part of scutellarin in the wastewater. If the scutellarin could be recycled, it can make full use of medicinal resources and reduce waste emission. However, few literatures have been reported. In this paper, we presents a simple method to retrieve high-purity scutellarin from the waste liquid for the purpose of improving the utilization of medicinal herb.

EXPERIMENTAL SECTION

Materials

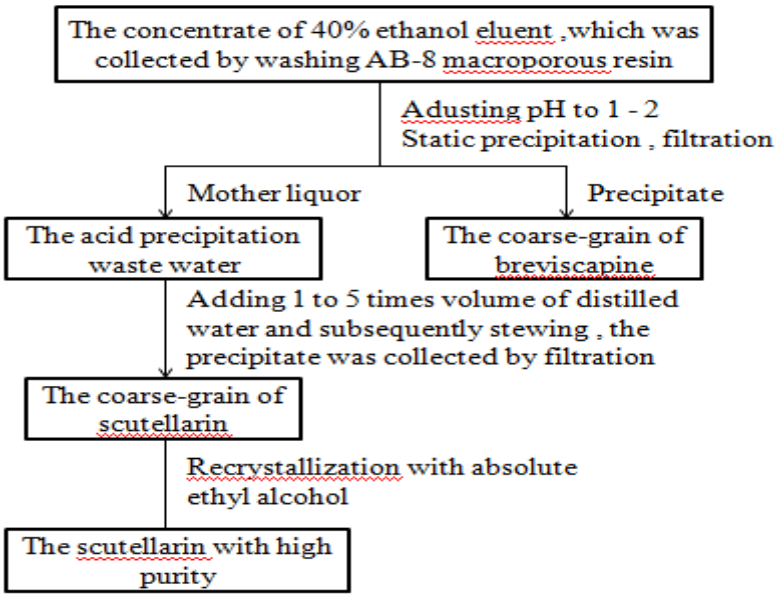
The HPLC system was Agilent-1200 (the company of American Agilent, equipped with auto-sampler, quaternary

pump, column heaters, a diode array detector, chromatography workstation); the mass was weighted by an electronic analytical balance (Shanghai Ohaus Instruments Co. Ltd.); ultrasonic generator was used to extract (the frequency of operation is 40KHZ,the ultrasonic power is 100W).

E. breviscapus was purchased from the Juhuayun Medicinal Herbal Market in Kunming. It was identified by Dr. Xiang. Scutellarin standard (HPLC>98%) was purchased from Chengdu Ruifensi Biological Technology Co., Ltd. AB-8 macroporous adsorption resin (Tianjin Bohong resin technology Co. Ltd) was used. Methanol and acetonitrile were chromatographical pure (Jiangsu Hanbang Technology Co., Ltd.), absolute ethyl alcohol and hydrochloric acid was analytical-reagent grade (Tianjin Municipality kemi'ou Chemical Reagent Co., Ltd.).

METHODS

The craftwork for Retrieving Scutellarin from the Acid Precipitation Waste liquid is shown in scheme 1.



Scheme 1 The craftwork for retrieving scutellarin from the acid precipitation waste liquid

Extraction and enrichment flavonoids with macroporous resin

E. breviscapus was smashed and twelve times of 70% ethanol (w/v) was added into 400g powder (60 mesh), extracted with ultrasonic for 40min, such operation was repeated for three times. The extract solution was combined and concentrated subsequently until no alcohol was scented. The concentrate liquid was filtered and finally, 800ml filter liquor was collected.

The filtrate was adsorbed by AB-8 macroporous resin and eluted by 8 bed volumes (BV) of distilled water; followed with 4 BV of 40％ (v/v) alcohol . After the alcohol elution was collected and concentrated, 1200 ml solution without alcohol was obtained. Draw out 2.0ml extracted liquor and dry in vacuum, the dry substance was regarded as sample 1.

Acid precipitation

The content of scutellarin in waste liquid is different from different pH value. So, adding different amount of hydrochloric acid to 150 ml concentrated liquid to adjust pH value from 1 to 2 (n = 3), respectively. After static precipitation and filtration, the mother liquor was considered as acid precipitation waste liquid. Preparing 2.0ml waste liquid precisely and vacuum drying, the dry substance were regarded as sample 2 (waste liquid of pH1) and sample 3 (waste liquid of pH2).

Retrieving scutellarin from acid precipitation waste liquid

The waste liquid (acid precipitation at pH 1.0 and 2.0) were concentrated into thick liquid, added one times, three times and five times volume of distilled water (n =3). After static precipitating for 12 hours at 4℃, the precipitate was collected by filtration and washed with a little distilled water. The coarse-grain of scutellarin were got by vacuum drying the subside and numbered as samples 4～9 successively. The samples 4- 9 were recrystallized with absolute ethanol. Finally, the high purity scutellarin was obtained by vacuum drying and numbered as samples10～

15 successively .

The HPLC determination method

The contents of scutellarin were tested by HPLC-UV method. The chromatographic conditions were as follows, column was Agilent ZORBAX SB-C18 (4.6 × 250 mm, 5 μ m) ; the mobile phase consisted of acetonitril (A) and 0.1% phosphoric acid (B) at a gradient of 3:17~2:3 (*v*/*v*) in 15 minute; the flow rate was at 0.8mL/min ; the column temperature was 30 ° C; the UV detector was at 335 nm; and the injection volume was 10 μ L.

The verification of the determination method has been done. The scutellarin stock solution was at 105 μg/ml. A series standard solution at 105 μ g/ml, 84 μ g/ml, 63 μ g/ml, 42 μ g/ml, 21 μ g/ml, 10.5 μ g/ml, and 5.25 μ g/ml were obtained by diluting the stock solution with methanol and detected by HPLC. The concentration C (g/ml) of reference substance was used as abscissa, while the peak area Y was used as ordinate, the working curve of scutellarin was Y= 30.389c +3.8889 (r=0.9998).The result shown that the linear relationship was good between 5.25 μ g/ml and 105 μ g/ml. Further study indicated that the precision (RSD) was 0.65%, the repetitive (RSD) was 0.53% , and the recovery rate was 99.63%～103.21% at 105 μ g/ml, 42 μ g/ml, 5.25 μ g/ml. The sample was stable within 24h with the RSD at 0.16%, The determination method was reliable and applicable.

The content of scutellarin in samples

The extract or precipitation samples were weighed precisely, dissolved in methanol (concentrations were shown in Table 1), filtered through 0.45 μM membrane and detected by HPLC. The content of scutellarin was measured and the purity was count as follows,

The content of scutellarin (%) = concentration of scutellarin / concentration of extract (precipitation) × 100%

RESULTS AND DISCUSSION

The concentration and content of scutellarin in each samples were shown in table 1. The purity of precipitate was higher when adding five times volume of water (around 60%). After recrystallization, the purity reached 90% (Figure 2).

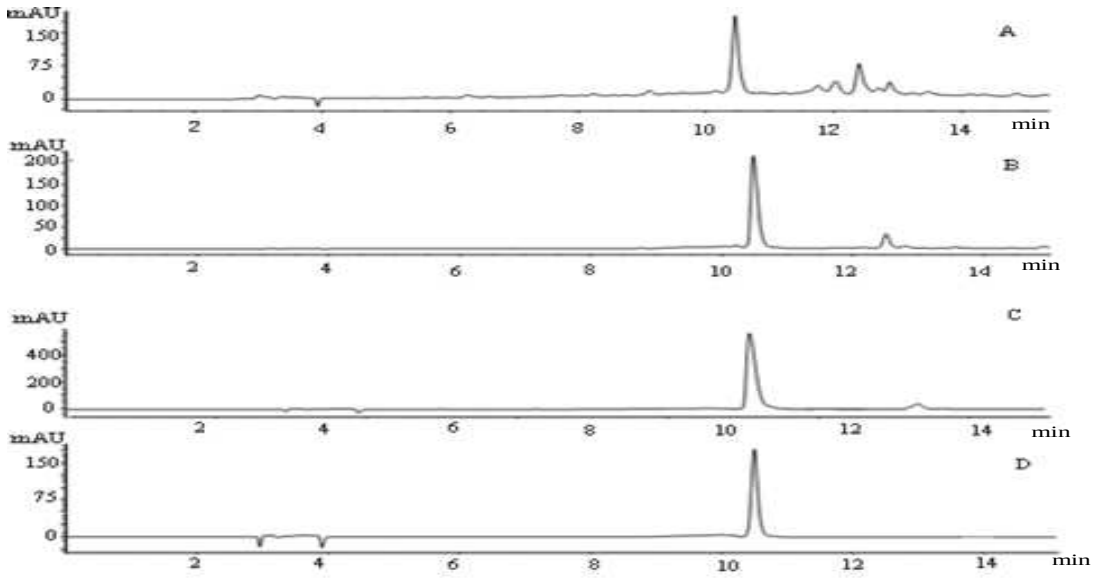


Fig 1 Retrieving scutellarin from the acid precipitation waste liquid (pH 2.0). The chromatograms were detected by HPLC-UV method at 335nm. A）the acid precipitation waste liquid (pH 2.0); B）the coarse-grain of scutellarin, obtained after adding 5 times volum of distilled water (sample 9). C）the high purity scutellarin was obtained by recrystallization (sample 15). D）the reference substance of scutellarin

Table 1 The concentration and purity of scutellarin in samples (n=3)

Sample	Concentration of scutellarin (μg /ml)	Concentration of extractum (precipitation) (μg /ml)	Purity of scutellarin in extractum (precipitation) （%）	Illustration
1	21.7	100	21.7	The concentrate of 40% ethanol eluent
2	20.3	195	10.4	The acid precipitation waste liquid at pH1.0
3	15.35	89	17.2	The acid precipitation waste liquid at pH2.0
4	22.45	90	24.9	Adding 1 time volume of water in pH1.0 waste liquid
5	59.05	190	31.1	Adding 3 times volume of water in pH1.0 waste liquid
6	49.25	80	61.6	Adding 5 times volume of water in pH1.0 waste liquid
7	54.05	330	16.4	Adding 1 time volume of water in pH 2.0 waste liquid
8	23.5	80	29.4	Adding 3 times volume of water in pH 2.0 waste liquid
9	38.4	65	59.1	Adding 5 times volume of water in pH 2.0 waste liquid
10	79	105	75.2	The elaboration product of sample 4
11	128.8	146	88.2	The elaboration product of sample 5
12	103.6	115	90.1	The elaboration product of sample 6
13	76.15	104.5	72.9	The elaboration product of sample7
14	80.2	90	89.1	The elaboration product of sample 8
15	110.05	122	90.2	The elaboration product of sample 9

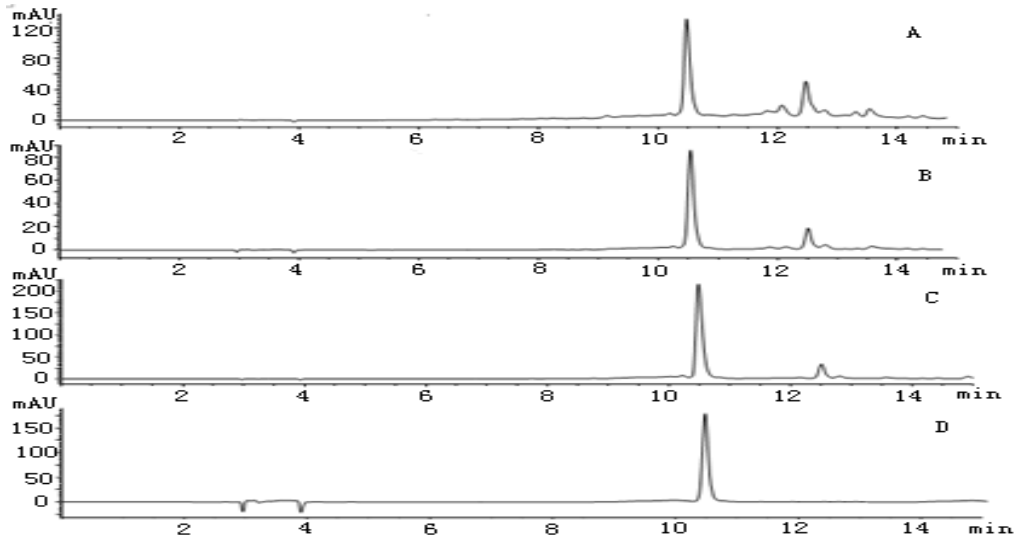


Fig 2 The volume of diluting water influenced the purity of scutellarine retrieved from the acid precipitation waste water. The waste liquid at pH 2.0 was diluted by different times of water. A）one times volume of water ; B）three times volume of water ; C）five times volume of water ; D）the scutellarin standard

DISCUSSION

pH value of acid precipitation influenced the purity of scutellarine

Acid precipitation is a key step in the process of preparing scutellarin, The pH value must be within 1～2. If the pH value was higher than 3, there would be no precipitation. This point was certificated by literature [3] and our preliminary experiment. When the pH value was at 1.0, the amount of precipitate was larger, however, the purity was relatively lower (23%), and the content of scutellarin in waste liquid was also lower (10.4%). In contrast, although the yield of precipitate was fewer when the pH value was 2.0, the precipitate purity was higher (84%), and the content of scutellarin in waste liquid was also higher (17.2%). So, the pH value should be at 2.0 in acid precipitation process as same as the report [7].

The volume of diluting water influenced the purity of scutellarine

In preliminary experiment, water was accidently sucked into the acid precipitation waste liquid and a yellow precipitation was separated out. This precipitate was identified as high purity scutellarin by comparing with standard in HPLC. In order to verify such phenomenon, one times, three times and five times volume of water was added to the slightly concentrated waste liquor respectively. Excitedly, the phenomenon reproduced times again, and the purity of scutellarin was higher as long as adding more water (Figure 2). After five times volume of distilled water was added, the purity was around 60%. However, if adding much water, the waste liquor will be too much to handle. So adding five times volume of distilled water is suit.

Possible mechanism

The mechanism of this phenomenon might be as follows, in the acid waste liquid, the scutellarin's hydroxyl formed complexes with hydrogen ions. The scutellarin exist as oxonium salt form. If adding distilled water, the oxonium salt will be unstable and decompose to original scutellarin. The water solubility of scutellarin is small, so it can be separated out as precipitate.

CONCLUSION

After adding five times volume of distilled water and following recrystallization, high purity scutellarin (more than 90%) was retrieved from the acid precipitation waste liquid. This simple method improves the utilization of medicinal materials and the cost is low, could be used in the industry.

Acknowledgements

The authors gratefully acknowledge the financial support from the Scientific Research Fund Projects in department of education of Yunnan (Grant NO. 2013Y324), the Leading Academic Discipline Project of Kunming University of Science & Technology (NO. 14078184).

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