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Pilot-Scale Multi-Stage Countercurrent Extraction of Scutellarein from *Erigeron breviscapus* (Vant.) Hand-Mazz

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Abstract: A low-temperature, timesaving, lower solvent consumption, and energy cost and multi-stage countercurrent extraction (MCCE) technique was developed for pilot-scale production of scutellarein from *Erigeron breviscapus* (Vant.) Hand-Mazz. The optimum conditions of MCCE process were obtained using the orthogonal array design method, i.e., 70% (v/v) of ethanol water solution, 16 L/kg of solvent to herbal sample ratio, 45°C of extraction temperature and 30 min of extraction time. A comparison between the MCCE technique and single pot extraction (SPE) under respective optimized operation conditions was made and time courses of scutellarein of MCCE and SPE processes were plotted, indicating that the MCCE technique can lower the extraction temperature and decrease five-sixth of the extraction time and two-thirds of the solvent consumption at the equivalent extraction yield of scutellarein.

Keywords: *Erigeron breviscapus*, herbal medicine, multi-stage countercurrent extraction, pilot scale, scutellarein, traditional Chinese medicine

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INTRODUCTION

Erigeron breviscapus (Vant.) Hand-Mazz (EBHM), a herb belonging to the Compositae family, has been used in China over centuries for preventing blood coagulation, enhancing blood circulation and dilating brain blood vessels, evidenced by its clinical application in the treatment of brain thrombosis and associated paralysis, myocardial ischemia, and coronary arteriosclerosis (1–4), especially as a long-range therapeutic drug with no evident side effects (5). Researches indicate that EBHM mainly contains flavonoids, phenolic acids, and their glucuronides, etc. Among these, scutellarein is one of the most important active constitutes (see Fig. 1) (6). Conventional extraction methods from EBHM are single pot extraction (SPE) and the percolation extraction (PE), both laborious, solvent consumptive, and poor extraction yield (7,8). In recent years, some novel methods such as microwave-assisted extraction (MAE), ultrasonic extraction (UE), and supercritical fluid extraction (SFE) have become the research hotspot in the field of herbal industry. Despite the advantages of short extraction time, relatively mild extraction and high extraction yield, the economics and onerous operating conditions of the SFE processes has restricted the applications to some very specialized fields such as essential oil extraction or to university research. Scaling-up strategy of MAE and UE technology is still not feasible. These facts result in the limited commercial application of SFE, MAE, and UE in the herbal industry. Another attractive alternative emerging recently is multi-stage countercurrent extraction (MCCE) which combines circulatory dynamic extraction and continuous countercurrent extraction technology (9). Studies on MCCE have been reported as early as 1980 and since then both the processing technique and equipment have been developed continuously (10). Applications of MCCE for extracting bioactive

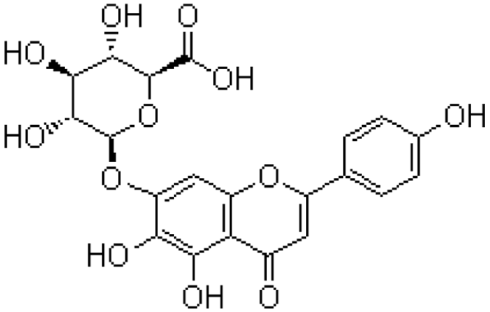


Figure 1. Molecular structure of scutellarein.

compounds from herbal samples have also been evaluated in some literatures such as the extraction of dihydromyricetin from *Ampelopsis grossedentata* (11), glycyrrhizic acid from licorice (*Glycyrrhiza uralensis* Fisch) (12) and polyphenols from green tea (13).

The present work described a study on pilot-scale extraction of scutellarein from EBHM with MCCE technology. The effects of important technical parameters on extraction yield were investigated, allowing the choice of the best extraction conditions to obtain a scutellarein-enriched extract. The dynamic course of scutellarein concentration in MCCE process was analyzed and compared with the SPE process, considering the extraction yield and energy cost.

EXPERIMENTS

Apparatus and Materials

A 10-liter MCCE equipment was designed and manufactured by our laboratory. A schematic diagram of the system employed in this study was shown in Fig. 2. A Waters HPLC equipped with Waters 2996 detector was used for scutellarein determination. EBHM materials were bought from Guizhou province, southwest China.

Standard scutellarein was purchased from the National Institute for the Control of Pharmaceutical and Biological Products. Ethanol (95%, v/v) used as the extraction solvent was of medical grade. Acetonitrile with chromatographically pure grade was purchased from Merck for HPLC analysis. All other reagents were of analytical grade. Water used

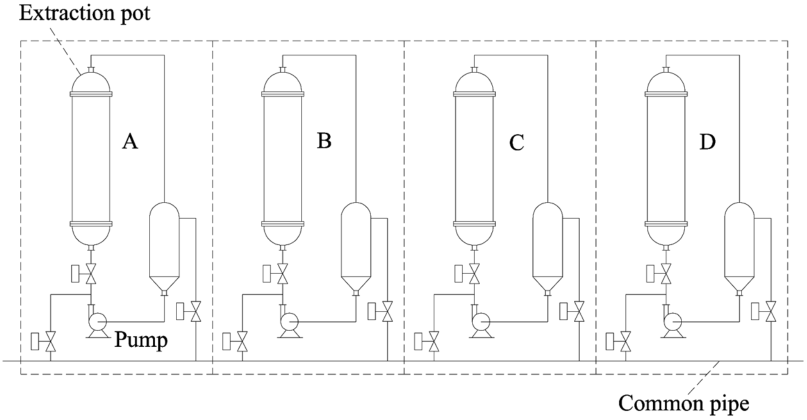


Figure 2. Pilot-scale MCCE equipment.

as the mobile phase in HPLC analysis was prepared by Millipore water purification device (Millipore, USA) in our laboratory.

Procedure

Multi-Stage Countercurrent Extraction

Extractions were accomplished using the pilot-scale MCCE equipment with four 10-liter extraction units (A, B, C, D). Each unit consisted of an extraction pot, a buffer pot, a pump, and three valves of the same configuration and dimensions. The extraction units were connected by pipes that were equipped with filtration sets for filtrating solid substance and preventing pipe jam. In a MCCE process, the solvent was pumped through the bottom valve to the pot, mixed with the herbal samples and then exited from the top of the pot and back to the pump.

The operation procedure of MCCE was referred to the instructions illustrated in Fig. 3. The symbols circle (◦) and square (□) represent powdery EBHM samples and extracts, respectively. The number (from 0 to 4) of disks (●) represents the scutellarein concentration in the matrix or the extract. For example, fresh solvent was expressed as a circle without disks and the symbol of the crude powdery EBHM was a square with 4 disks inside. ⇨ and ⇦ mean pumping the extract from the extraction pot to the storage pot and unloading the herbal sample slurry from the extraction pot, respectively. Fresh solvent was denoted as symbols of a–g. Processes 1–4 in Fig. 3 illustrated the transference process between the solvent and the herbal matrices in each of the sequential steps. There were four basic operations in every process. For example, Process 1 included: (a) extracting herbal samples for a predetermined time, (b) unloading herbal sample slurry from extraction pot A and collecting the extract from extraction pot D, (c) transferring solvents in the directions of B → D, C → B, A → C, B → A and (d) adding herbal samples and fresh solvent into units A and B, respectively.

For a whole cycle operation of MCCE, about 250 g of powdery EBHM sample was added into an extraction pot and mixed with about 750 ml of solvents for 20 min, and then MCCE proceeded continuously from Process 1 to Process 4.

Single Pot Extraction

Single pot extractions, optimized in advance, were carried out by using an extraction pot of the same equipment. 250 g of the powdery EBHM

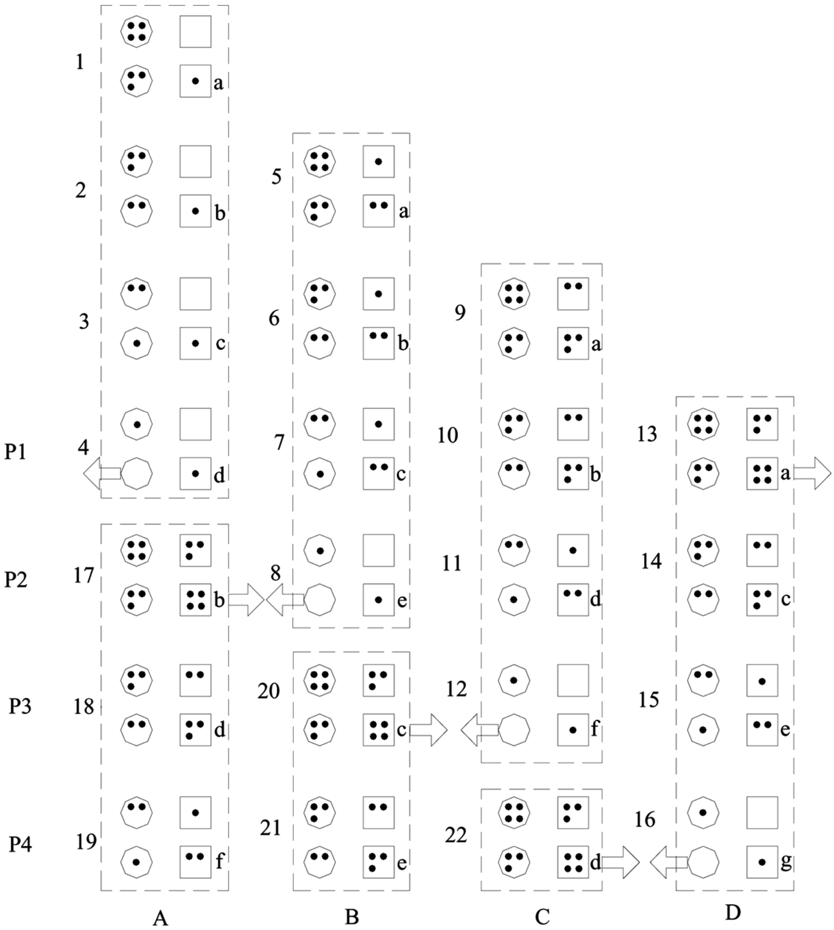


Figure 3. Flow diagram of MCCE process.

sample was soaked in 750 ml of ethanol (70%, v/v) for 20 min at room temperature and then more 4 L of the ethanol was added. The SP extraction lasted 60 min at 75°C and repeated thrice.

Analytical Procedure

Scutellarein concentration in extracts was determined by a validated reverse-phase HPLC assay. All determinations were performed at 30°C using a Hanbon Lichrospher C_{18} column (4.6 mm × 250 mm, 4 μm).

Chromatographic separation was conducted with a gradient elution. The elution gradient consisted of two solvent compositions: 0.1% acetic acid water solution (solvent A) and 0.1% acetic acid acetonitrile solution (solvent B). A linear gradient was programmed as follows: solvent A was decreased from 90% to 80% in the first 30 min, then decreased to 20% in the next 1 min, held for 4 min and then returned to 90% in 1 min. The elution was monitored by a UV detector at the wavelength of 335 nm. The flow rate was constant at 1.0 ml/min.

Under the above conditions, a linear calibration line of peak area (A, AU) versus scutellarein amount (m, μg) was obtained in the range of 2.6∼26.0 μg of scutellarein with a regression equation of $A = 3.25\, m + 0.09$ and a correlation coefficient $r = 0.9996$. The relative standard deviation of scutellarein measurement was 1.78% ($n = 5$).

RESULTS AND DISCUSSION

MCCE Process Optimization

In general, ethanol concentration in water (c, v/v), solvent to powdery herbal sample ratio (n, L/kg), extraction temperature (T, °C), and extraction time (t, min) were regarded as the main factors impacting on the extraction yield of MCCE process (14).

In this study, an orthogonal array design was applied to optimize the experimental conditions with as few experiments as possible (15). Three levels were selected for each variable and a $L_9(3^4)$ table was used to arrange the experiments (see Table 1). MCCE processes under different operation conditions were gauged by an indicator of the extraction yield of scutellarein in this study. The experimental results were also listed in Table 2.

Table 3 summarized the analysis results of variance that showed ethanol concentration had a strong impact on MCCE yield of scutellarein as indicated by the high F-value of 55.4. When the ethanol concentration was changed from 50% to 70%, the Scutellarein concentration was

Table 1. Factors and levels of $L_9(3^4)$ orthogonal design

	Factors			
Levels	C (%)	n (L/kg)	T (°C)	t (min)
1	50	8	30	30
2	60	12	45	45
3	70	16	60	60

Table 2. Arrangement and results of orthogonal design

Run	Factors				Scutellarein yield (g/kg)
	c	*n*	*T*	*t*	
1	1	1	1	1	1.76
2	1	2	2	2	3.74
3	1	3	3	3	4.39
4	2	1	2	3	5.21
5	2	2	3	1	5.11
6	2	3	1	2	4.79
7	3	1	3	2	6.27
8	3	2	1	3	5.28
9	3	3	2	1	6.95
L_1	3.30	4.41	3.94	4.61	
L_2	5.04	4.71	5.30	4.93	
L_3	6.17	5.38	5.26	4.96	
ΔL	2.87	0.96	1.36	0.35	

increased 87%. Extraction temperature was another significant factor for MCCE yield of scutellarein. The extraction yield was increased as temperature increased and reached a maximum value near 45°C, and then slightly decreased with the further increase in temperature. Meanwhile, as the ratio of solvent to sample changed from 8 L/kg to 16 L/kg, the extraction yield would be increased 22%. Poor improvement in scutellarein extraction yield was gained even when the extraction prolonged from 30 min to 60 min. Based on these analysis results, the optimal conditions of MCCE of scutellarein was obtained with $c_3n_3T_2t_1$, in which c_3 denoted the third level of 70% for ethanol concentration in water, n_3 denoted the third level of 16 L/kg for solvent to sample ratio, T_2 denoted

Table 3. Variance analysis of the orthogonal experiment

SV	SS	DF	MS	*F*-value	Significance
c	12.54	2	6.27	55.4	++
n	1.46	2	0.73	6.3	
T	3.57	2	1.78	15.4	+
t	0.23	2	0.12	1.0	
Error	0.23	2	0.12		
Total	18.03	8	9.02		

SV: source of variance, SS: sum of squares, DF: degrees of freedom, MS: mean square. ++: $F_{0.05}$ (2,2) = 19.00, +: $F_{0.1}$ (2,2) = 9.00.

the second level of 45°C for extraction temperature, and t_1 denoted the second level of 30 min for extraction time.

Under the optimized MCCE conditions, scutellarein extraction was carried out in triplicate in the MCCE equipment. The average extraction yield of scutellarein was 7.08 g/kg.

Comparison of Different Extraction Methods

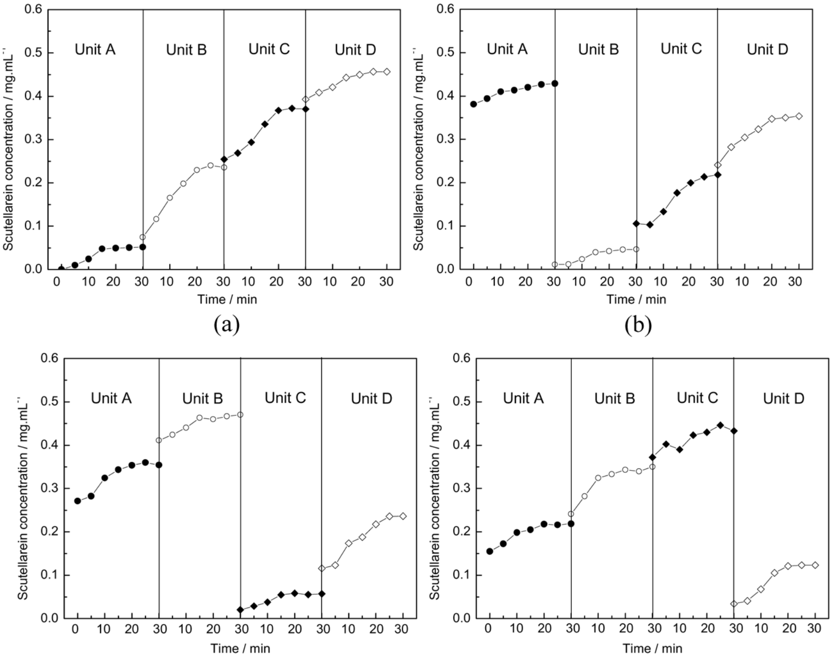
A comparison between MCCE and SPE was made in terms of scutellarein extraction yield per unit weight of herbal sample and solvent consumption per unit weight of herbal matrix. The experimental result was shown in Table 4. Although the extraction yield of scutellarein from MCCE was slightly lower than that of the triple SPE operation, MCCE offered advantages with respect to extraction time and solvent consumption. For the same weight of herbal samples, it took only one-sixth of extraction time and one-third of solvent as the triple SPE. Moreover, the SPE technique had the extraction temperature of 75°C higher than MCCE process. Compared with the triple SPE operation, the single or double SPE operation resulted in substantial decrease of time and solvent consumption. However, that resulted in dramatic drops of extraction yield, as Table 4 showed.

It is known that mass transfer from the matrix surface to the liquid phase is resulted mainly from the diffusion from one region to another region of different concentration. Fick's first law for steady-state condition states that the mass transfer flux by ordinary molecular diffusion is equal to the product of the diffusion coefficient (diffusivity) and the negative of the diffusion concentration gradient. As described above, MCCE

Table 4. Comparison between SPE and MCCE

	SPE-1*	SPE-2	SPE-3	MCCE
Amount of herbal samples (kg)	0.25	0.25	0.25	1.75
Extraction temperature (°C)	75	75	75	45
Time consumption per unit weight of herbal samples (min/kg)	240	480	720	120
Scutellarein yield per unit weight of herbal samples (g/kg)	2.58	4.82	6.23	6.11
Solvent consumption per unit weight of herbal samples (L/kg)	16	32	48	16

*SPE-1: single SPE operation, SPE-2: double SPE operation, SPE-3: triple SPE operation.



(a) (b) (c) (d)

Figure 4. Time course of scutellarein concentration during various processes of MCCE.

was carried out by creating a concentration difference between the solvent and solid phases. Figure 4 showed the dynamic course of scutellarein concentration in four extraction units (A, B, C, D) by stages in MCCE process. It is easy to notice that the concentration was enhanced continuously, except in the forth extraction stage for each batch during which it was slightly increased at first and then reached a plateau state. This result suggested that 105 min was enough to extract scutellarein successfully from *Erigeron breviscapus* by the optimized MCCE technique.

Similarly, the experimental results of SPE in unit A were shown in Fig. 5. In the first process of SPE, the scutellarein concentration was increased quickly at least in the first 30 min. Then, in the second process, it still could be increased steadily, but extraction was far lower than before. In the third one, the scutellarein concentration only grew slowly in 30 min and then kept steady, indicating that the extraction was finished thoroughly. In at all, about 150 min was needed for a batch of SPE to extract scutellarein from the herbal samples, more time-consuming than MCCE process.

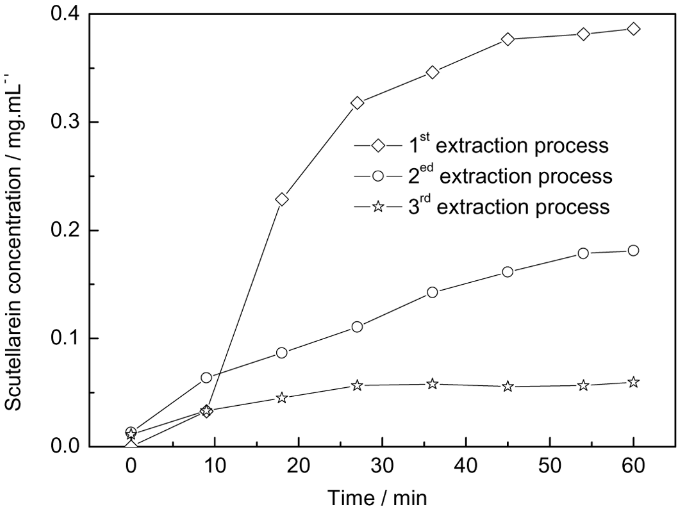


Figure 5. Time course of scutellarein concentration during SPE process.

The above facts indicated that SPE needs longer extraction time, higher extraction temperature, and more extraction solvents to obtain the equivalent amount of scutellarein than MCCE. Higher solvent consumption per unit weight of herbal sample, meaning lower scutellarein concentration in extracts, would inevitably result in more energy cost for the subsequent concentration process of the extracts. To sum up, MCCE is a timesaving, energy-saving, and cost-saving, extraction technology for manufacturing of the herbal medicines.

CONCLUSION

Current extraction methods widely applied in the herbal industry, such as SPE, suffer from high solvent and energy consumption and low extraction yield. As proved to be timesaving, lower solvent consumption, and energy cost, the proposed MCCE technique provides an attractive alternative to solve the above problems. The optimized MCCE process conditions for scutellarein extraction with 70% of ethanol, 16 L/kg of solvent to herbal sample ratio, 45°C of extraction temperature, and 30 min of extraction time has been obtained using the orthogonal array design method. Comparison between MCCE and SPE show that MCCE does have overwhelming advantages in lower extraction temperature, shorter extraction time, lower solvent consumption, and energy cost for manufacturing of scutellarein from EBHM.

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