



# On-line coupling of dynamic microwave-assisted extraction with high-speed counter-current chromatography for continuous isolation of nevadensin from *Lycionotus pauciflorus* Maxim.

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## ABSTRACT

An on-line method based upon dynamic microwave-assisted extraction (DMAE) coupled with high-speed counter-current chromatography (HSCCC) was developed for continuous isolation of nevadensin from *Lycionotus pauciflorus* Maxim. The DMAE parameters were optimized by means of the Box–Behnken design. The maximum extraction yield was achieved using 30:1 ml/g of liquid–solid ratio, 10 ml/min of solvent flow rate and 200 W of microwave power. The crude extracts were then separated by HSCCC with a two-phase solvent system composed of n-hexane–ethyl acetate–methanol–water (7:3:5:5, v/v/v/v). 13.0 mg of nevadensin was isolated from 15.0 g original sample by HSCCC with five times sample injection in 12 h, and the isolation yield of nevadensin was 0.87 mg/g. The average purity of nevadensin was higher than 98.0%. The chemical structure of collected fraction was identified by HPLC, ESI-MS and <sup>1</sup>H NMR. The results indicated that this on-line method was effective and fast for high-throughput isolation of nevadensin from *L. pauciflorus* Maxim.

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## 1. Introduction

*Lysionotus pauciflorus* Maxim. (Chinese name: Shidiaolan) was species of Gesneriaceae genus of *Lysionotus*, and it was an important crude herb used in traditional Chinese medicines (TCMs). It has been proven to be effective in the treatment of lymph node tuberculosis, cough with tachypnoea and rheumatic pains [1,2]. Nevadensin (5,7-dihydroxy-6,8,4'-trimethoxyflavone, Fig. 1) with good antibacterial, anti-inflammatory, antihypertensive and free radical-scavenging activities effects [3–5] was the major active constituent of *L. pauciflorus* Maxim. Therefore a high-throughput method for the isolation of nevadensin was required.

Several methods have been developed to analyze nevadensin in *L. pauciflorus* Maxim. [3–5]. In these studies, maceration at room temperature [4,5], heating reflux extraction [6] and ultrasonic-assisted extraction [7] were used for extraction of nevadensin from *L. pauciflorus* Maxim. Those methods were time-consuming and low efficient. Microwave assisted extraction (MAE), which could provide good extraction efficiency and large treating amount, was used as an alternative method for the effective extraction of various natural products [8–10]. MAE could be performed by either static or dynamic mode. In the static MAE, the sample was extracted in

a closed-vessel system, and the recovery of targeted component mainly relied on its rate constant of desorption in the extraction solvent [11]. In recent years, the dynamic MAE (DMAE) was gradually developed, and it could continuously supply the fresh extraction solvent to the extraction vessel [12–18]. Moreover, the DMAE suggested a possibility of the automation in the sample isolation step [19].

Although DMAE extraction method was fast and had a higher extraction yield, the next separation processes, such as silica gel, polyamide and preparative HPLC, were tedious, time-consuming, and required multiple chromatographic steps [20]. As a support-free liquid–liquid partition chromatographic technique, HSCCC has been widely used to separate natural products from medicinal plants. Compared with traditional separation methods, HSCCC eliminated the complications coming from the solid support matrix, such as irreversible adsorptive sample loss, deactivation, tailing of solute peaks and contamination [21–23]. Recently, the supercritical fluid extraction (SFE) [24–26] and MAE [8–10] have been used as independent pretreatments on the extraction of target compound before it was separated by HSCCC. However, there were no reports on the isolation of target compounds by on-line coupling DMAE with HSCCC.

The main purpose of this study was to develop an on-line method to improve and simplify the isolation of nevadensin from *L. pauciflorus* Maxim. by coupling DMAE with HSCCC. The purity of obtained nevadensin was determined with HPLC and its chemical structure was identified with ESI-MS and <sup>1</sup>H NMR.

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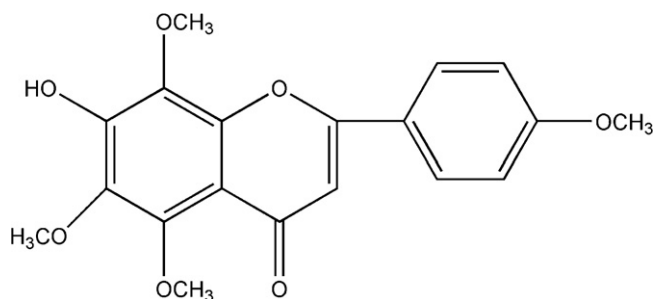


Fig. 1. The chemical structure of nevadensin.

## 2. Experimental

### 2.1. Chemicals and samples

Standard nevadensin was purchased from Guangdong Institute for Drug Control (Guangzhou, China). Dryness *L. pauciflorus* Maxim. was purchased from the Caizhiling medicinal material emporium in Guangzhou (Guangdong, China). Chromatography grade acetonitrile was purchased from Merck (Darmstadt, Germany). All the other organic solvents of analytical grade were purchased from Guangzhou Chemical Factory (Guangdong, China). Stock solution of the standard (52 µg/ml) was prepared by dissolving nevadensin in acetonitrile. They were stored in a refrigerator at 4 °C.

### 2.2. Apparatus

The on-line DMAE-HSCCC system composed of extraction, concentration and separation module was self-designed and constructed by our laboratory (Sun Yat-sen University, Guangzhou, China). The schematic diagram was shown in Fig. 2. The three modules were connected by some peristaltic pumps (pump 1–5 in Fig. 2, Jieheng, Chongqing, China) and polytetrafluoro ethylene tube (3.0 mm ID × 5.0 mm OD) (Longer, Baoding, China). The microcomputer (89E516RT, SST, China) was used to automatically control all pumps and valves in the on-line system.

The extraction module mainly performed on a MAS-II microwave oven from Sineo Microwave Chemistry Technology Company (Shanghai, China) with a frequency magnetron of 2450 MHz and a maximum output power of 1000 W. It was an open system equipped with a temperature and power feedback control. The temperature was monitored by an infrared probe inside the microwave oven.

The concentration module with an auto concentrator and a temperature transducer was constructed by our laboratory, and included an AutoScience AP-02B vacuum pump (Tianjin, China) and an IKA basic-2 magnetic force stirrer (Deutschland, Germany).

The HSCCC used in the separation module was a GS10A (Beijing UE Biotech., Beijing, China) equipped with PTFE multilayer coil (110 m × 1.6 mm ID, 224 ml). A manual sample injection valve (valve 1 in Fig. 2) with sample loop equipped in GS10A was modified to be an automatic sample injection valve by adding electric motor (TH37JB555, Tianheng Company, China), and it used to introduce the sample into the coil automatically. Furthermore, the solvent was pumped into the column with a Model NS-1007 constant-flow pump (pump 6 in Fig. 2, Beijing UE Biotech., Beijing, China). The out flow of HSCCC was detected by an 8823A-UV Monitor (Beijing UE Biotech., Beijing, China) at 280 nm and recorded by a HW-2000 chromatography workstation (Shanghai, China), then collected by a BSZ-100 fraction collector (Shanghai, China).

### 2.3. On-line coupling of DMAE with HSCCC

5 g samples of *L. pauciflorus* Maxim. were accurately weighted and then put into the extraction vessel. 50 ml methanol was pumped into the vessel first for immersion of the samples. Then the microwave oven was started with the power of 200 W. At the same time, the pump 1 and the pump 2 were simultaneously activated and the extraction solvent (methanol 150 ml) was passed through extraction vessel with a flow rate of 2.0 ml/min. The extraction was completed when the 200 ml extraction solvent all pumped into the auto concentrator. Then 5 g fresh original samples were put into extraction vessel for the next time extraction.

When the 600 ml of extraction solvents was condensed to dry extracts at 50 °C in auto concentrator, the dry extracts were dissolved with the 50 ml lower phase of the HSCCC solvent system of hexane–ethyl acetate–methanol–water (7:3:5:5, v/v/v/v) which pumped from the mobile phase bottle 1 by pump 3. The most of dry extracts dissolved in the lower phase were transferred to sample bottle for storage and then introduced into sample loop of automatic sample injection valve. When the sample injection was complete, the pump 6 started to run and the samples in sample loop were introduced into the HSCCC column by switching valve 1. After 20 min, the samples were completely pushed into the HSCCC column and the valve 1 was switched back to initial state for getting ready for next sample injection. The effluent from HSCCC was monitored at 280 nm. After HSCCC separation, the collected fractions were analyzed by HPLC.

Briefly, the isolation yield of nevadensin was defined as following:

$$\text{Isolation yield (\%)} = \frac{\text{Mass of nevadensin obtained in isolation}}{\text{Mass of the original samples}} \times 100\%$$

### 2.4. The selection of the solvent system

The selection of the solvent system for separation of the target compounds was the most important step in HSCCC. The solvent system for HSCCC separation was selected according to the difference of partition coefficients (*K*) of each target compound between the two-phase systems. The *K* value was determined as follows: two-phase solvent systems with different ratios of organic solvent and water were prepared. Upper and lower phases (2 ml each) were placed in test tubes and 1 mg dry extracts added. Each test tube was shaken for 1 min and then left for the phases to separate. 500 µl of each phase was removed and concentrated to dryness. The residue was re-dissolved in 1 ml methanol and 10 µl analyzed by HPLC with UV detection at 280 nm. The *K* value was defined as the concentration of nevadensin in the upper phase (*C<sub>U</sub>*) divided by the concentration of nevadensin in the lower phase (*C<sub>L</sub>*) at the same retention time in the HPLC chromatogram, that was  $K = C_U/C_L$ . Furthermore, a successful separation in HSCCC largely depended on the separation factor  $\alpha$ , which was defined as  $\alpha = K_{\text{nevadensin}}/K_{\text{impurity}}$ .

The solvent systems of HSCCC were prepared with the appropriate solvent volumes. Each solvent mixture was thoroughly equilibrated in a separation funnel for more than 12 h at room temperature.

### 2.5. HPLC analysis and identification of HSCCC fraction

A Shimadzu LC-2010 system (Tokyo, Japan) with UV detector was used for the analysis of the extracts of *L. pauciflorus* Maxim. The fractions and the collected nevadensin were filtrated through a 0.45 µm micro porous membrane. Chromatographic separation was performed on a Diamonsil C18 column (200 mm × 4.6 mm ID, 5 µm) equipped with an EasyGuard C18 guard column (10 mm × 4.6 mm ID) at 25 °C. The conditions of HPLC analy-

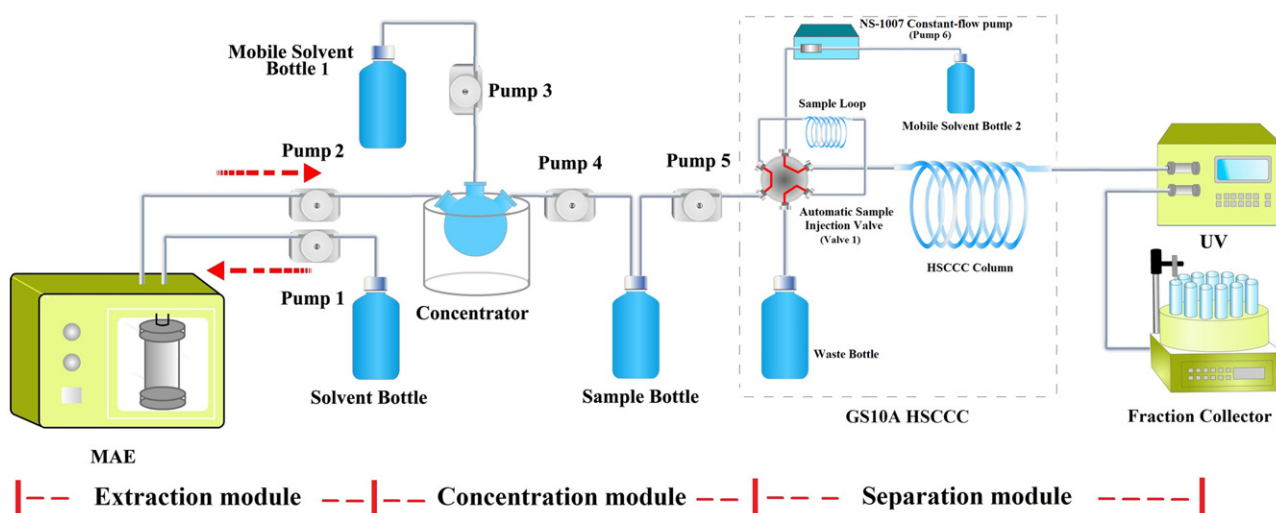


Fig. 2. The schematic diagram for on-line coupling DMAE with HSCCC.

sis for nevadensin were as follows. The mobile phase consisted of acetonitrile–0.5% acetic acid (60:40, v/v). The flow rate was 1.0 ml/min, the injection volume was 10  $\mu$ l and the detection wavelength was set at 284 nm. The purity of nevadensin obtained from the coupling method was also identified with ESI-MS (ThermoFisher, Waltham, USA) and Nuclear Magnetic Resonance (NMR) spectrometer (Mercury-plus 300, Varian, USA).

A series of standard solutions of nevadensin were prepared to determine the linearity of HPLC analysis. The linearity plotting at 284 nm was  $Y = 3.41 \times 10^4 X - 3.50 \times 10^4$  ( $R = 0.9991$ ) over the concentration range from 0.5 to 102.7 mg/l, where  $X$  was nevadensin concentration and  $Y$  was the peak area. The limit of detection was 0.013 mg/l which was evaluated on the basis of a signal-to-noise ratio of 3.0. The reproducibility was estimated by five repetitive samples extracted by DMAE at the optimum conditions. The relative standard derivation (RSD) of nevadensin was lower than 1.2%. The recovery of nevadensin for the spiked real samples was in the range of 94.1–103% with RSD lower than 2% based on the peak area for three replicates. The reproducibility and recovery proved that the analytical method in DMAE had good precision and accuracy.

Briefly, the extraction yield of nevadensin was defined as following:

Extraction yield (%)

$$= \frac{\text{Mass of nevadensin in extraction solvent}}{\text{Mass of the original samples}} \times 100\%$$

### 3. Results and discussion

#### 3.1. Optimization of DMAE conditions

##### 3.1.1. Effect of extraction solvents

The extraction solvent was an important factor which would significantly influence the extraction yield of nevadensin from *L. pauciflorus* Maxim. The effects of four conventional extraction solvents (water, methanol, ethanol and 50% ethanol) were investigated as follow: an amount of 1 g original sample was extracted by MAE with 30 ml different solvents for 10 min. The microwave power was 300 W and the extraction temperature was 50 °C. The results were shown in Fig. 3. The extraction yield obtained by using methanol was statistically higher than that by using other solvents, and methanol was apt to condense in subsequent concentration process. Thus methanol was used as extraction solvent in the work.

##### 3.1.2. Effect of DMAE conditions

The Box–Behnken design was a second-order multivariate technique and received a wide application for assessment of critical experimental conditions [27]. In this study, a 17-run Box–Behnken design was used to design the tests to explore the variables of DMAE that affect yield of nevadensin. The three independent variables used in the DMAE were the liquid–solid ratio ( $A$ ), solvent flow rate ( $B$ ) and microwave power ( $C$ ), while the response was the yield of nevadensin. For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and responses [28]. For the three factors, the equation was

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2$$

where  $Y$  was the predicted response (yield of nevadensin);  $A$ ,  $B$  and  $C$  were independent variables (liquid–solid ratio, solvent flow rate and microwave power);  $\alpha_0$  was model constant;  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_{12}$ ,  $\alpha_{13}$ ,  $\alpha_{23}$ ,  $\alpha_{11}$ ,  $\alpha_{22}$  and  $\alpha_{33}$  were linear coefficients.

All experiments were performed randomly to minimize the effects of uncontrolled factors that could have introduced bias into the measurements. Methanol was selected as extraction solvent. 5 g of original sample was used for all experiments. The experi-

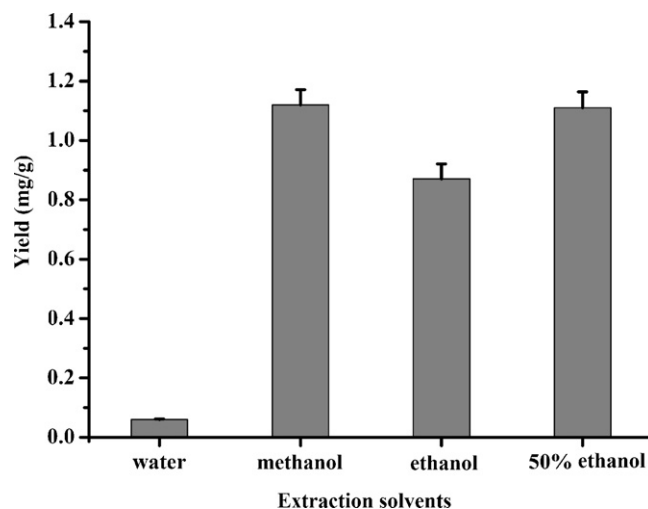


Fig. 3. Effect of different solvents used on the extraction yield of nevadensin.

**Table 1**  
Box–Behnken design matrix of independent variables and their corresponding responses.

Experiments	Coded levels			Responses: nevadensin yield (mg/g)
	A Liquid–solid ratio (ml/g)	B Solvent flow rate (ml/min)	C Microwave power (w)	
1	–1(10)	–1(10)	0(600)	1.03
2	+1(30)	–1(10)	0(600)	1.69
3	–1(10)	+1(30)	0(600)	0.75
4	+1(30)	+1(30)	0(600)	1.41
5	–1(10)	0(20)	–1(200)	0.89
6	+1(30)	0(20)	–1(200)	1.55
7	–1(10)	0(20)	+1(1000)	0.99
8	+1(30)	0(20)	+1(1000)	1.50
9	0(20)	–1(10)	–1(200)	1.47
10	0(20)	+1(30)	–1(200)	1.27
11	0(20)	–1(10)	+1(1000)	1.51
12	0(20)	+1(30)	+1(1000)	1.33
13	0(20)	0(20)	0(600)	1.35
14	0(20)	0(20)	0(600)	1.35
15	0(20)	0(20)	0(600)	1.39
16	0(20)	0(20)	0(600)	1.38
17	0(20)	0(20)	0(600)	1.44

**Table 2**  
Estimates of the model coefficients for the responses.

Coefficient	Estimate	P-values
$\alpha_0$	1.380	<0.0001
$\alpha_1$	0.310	<0.0001
$\alpha_2$	–0.120	0.0001
$\alpha_3$	0.019	0.2524
$\alpha_{12}$	0.000	1.0000
$\alpha_{13}$	–0.038	0.1211
$\alpha_{23}$	0.005	0.8208
$\alpha_{11}$	–0.160	0.0001
$\alpha_{22}$	0.000	0.9907
$\alpha_{33}$	0.013	0.5578

Coefficients of determination ( $R^2$ ) = 0.9876.

mental design was presented in Table 1, together with the obtained experimental responses studied in each experiment.

Experimental data analysis and quadratic model building were conducted using the Design Expert software (Trial Version 7.1.3, Stat-Ease Inc., USA). Model coefficients for each response were presented in Table 2. Generally the  $P$ -values less than 0.0500 indicated that model were significant [29]. In this case, the proposed mathematical models were significant ( $P$ -values of  $\alpha_0 < 0.0001$ ), and the liquid–solid ratio ( $A$ ) and solvent flow rate ( $B$ ) has significantly affected the yield of nevadensin ( $P$ -values of  $\alpha_1$ ,  $\alpha_2$  and  $\alpha_{11} \leq 0.0001$ ). In addition, coefficients of determination ( $R^2$ ) for extraction yields of nevadensin were 0.9876. The closer  $R^2$  to 1, the better the empirical model fits the actual data [30]. Therefore, the proposed models were accepted.

Surface response graphs, obtained by using the fitted model, were presented in Fig. 4. It was clear that the liquid–solid ratio ( $A$ ) and solvent flow rate ( $B$ ) have significantly affected the yield of nevadensin, and their effects were positive and nega-

**Table 3**  
The partition coefficient ( $K$ ) and separation factors ( $\alpha$ ) of different solvent systems.

Solvent systems (v/v)	Partition coefficient ( $K$ )		$\alpha$
	Impurity	Nevadensin	
Chloroform–methanol–water (2:1:1)	4.95	0.09	55.00
n-Hexane–methanol–water (2:1:1)	0.17	0.16	1.06
n-Hexane–ethyl acetate–methanol–water (1:1:1:1)	0.20	1.50	7.50
n-Hexane–ethyl acetate–methanol–water (8:2:5:5)	0.05	0.41	8.20
n-Hexane–ethyl acetate–methanol–water (7:3:5:5)	0.30	0.60	2.00
n-Hexane–ethyl acetate–methanol–water (6:4:5:5)	0.19	0.97	5.11
n-Hexane–ethyl acetate–methanol–water (5:5:5:5)	0.20	1.50	7.50
Ethyl acetate–water (1:1)	5.61	–	–

tive, respectively. The higher nevadensin yield was obtained when selected higher liquid–solid ratio or lower flow rate. The maximum extraction yield of nevadensin was achieved with 30:1 (ml/g) of liquid–solid ratio and 10 ml/min of solvent flow rate. The microwave power ( $C$ ) had not significant effect on nevadensin yield. The results also indicated that the interactions between the three parameters were not very significant.

By the established second-order polynomial model, the optimum DMAE conditions predicted by the software were 30:1 (ml/g) of liquid–solid ratio, 10 ml/min of solvent flow rate and 200 W of microwave power, and the actual yield of nevadensin was 1.61 mg/g.

### 3.2. Selection of HSCCC solvent system

A suitable solvent system for the successful HSCCC separation was important, which should have good separation capability to the target component. Although a series of rules have been presented and would rapidly guide the search for a suitable solvent system, the selection to successful solvent system was still very difficult. Overall, the partition coefficient ( $K$ ) was the most important parameter in solvent system selection, which should be in the range of 0.5–2.0 to get an efficient separation [31]. Based on the chemical properties of nevadensin that had hydrophobic and hydrophilic groups at the same time, several two-phase solvent systems, such as chloroform–methanol–water, n-hexane–methanol–water, n-hexane–ethyl acetate–methanol–water and ethyl acetate–water were tested and the  $K$  values were measured and summarized in Table 3.

These results indicated that ethyl acetate–water (1:1, v/v) had a large  $K$  value, while chloroform–methanol–water (2:1:1, v/v/v) and n-hexane–methanol–water (2:1:1, v/v/v) had smaller  $K$  values and

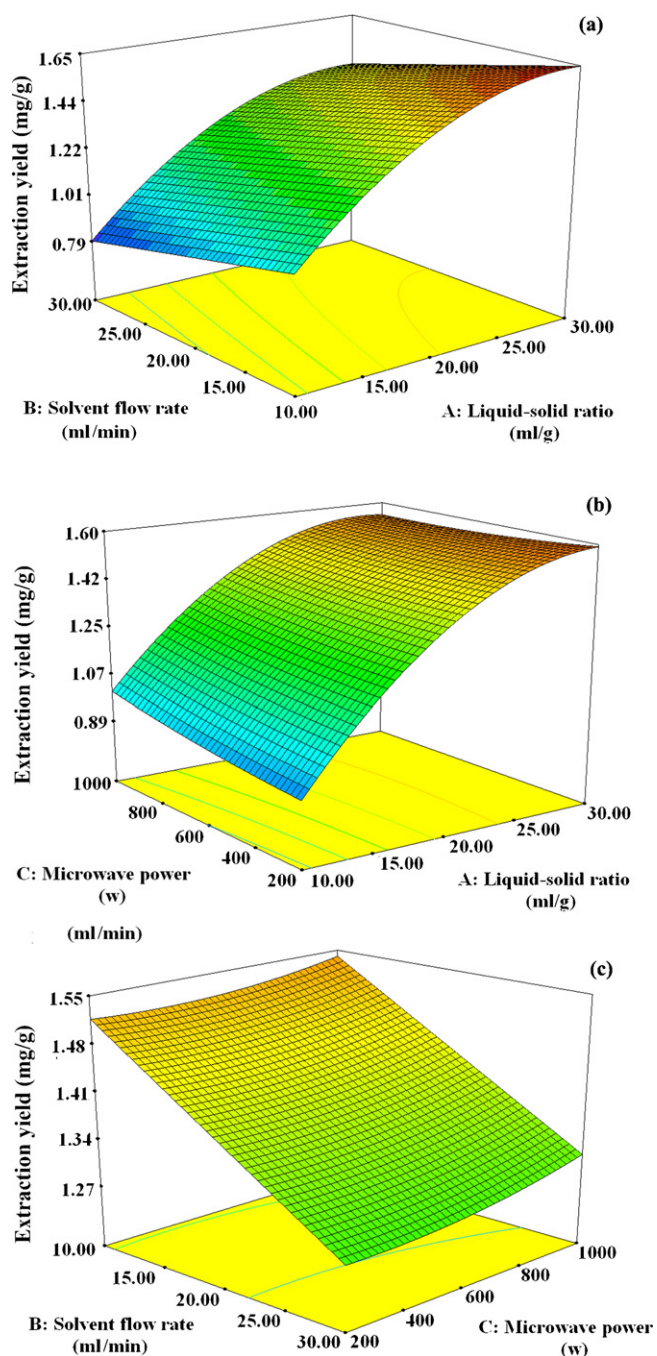


Fig. 4. 3D-surface plots showing the effects of: (a) liquid-solid ratio (ml/g) and solvent flow rate (ml/min); (b) liquid-solid ratio (ml/g) and microwave power (w); (c) solvent flow rate (ml/min) and microwave power (w).

that both were unsuitable for HSCCC separation of nevadensin from *L. pauciflorus* Maxim. Consequently, the solvent systems composed of n-hexane-ethyl acetate-methanol-water at different volume ratios (8:2:5:5, 7:3:5:5, 6:4:5:5, 5:5:5:5, v/v/v/v) were selected to study the optimum conditions. The HSCCC chromatograms were shown in Fig. 5. The suitable  $K$  value and the shorter consumed time were obtained when n-hexane-ethyl acetate-methanol-water (7:3:5:5, v/v/v/v) was selected as the two-phase solvent system.

### 3.3. Optimization sample loading of HSCCC

The maximum single-injection sample loading could cause stationary phase loss and poor purity of the isolated component. When the extracts were injected into the HSCCC system for separation,

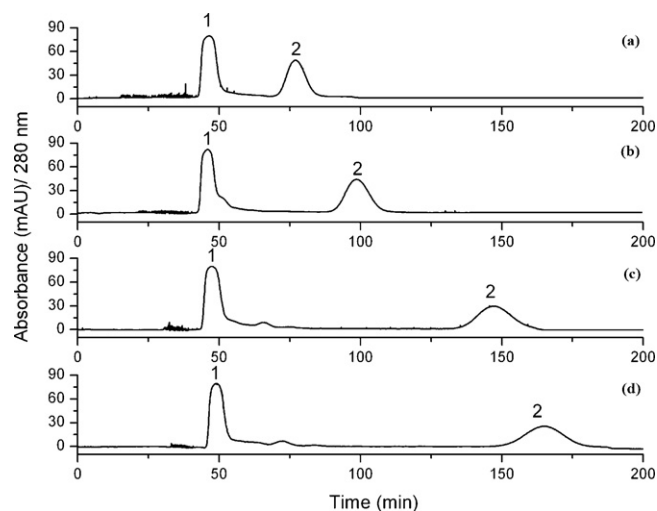


Fig. 5. The HSCCC chromatograms of isolation of nevadensin with n-hexane-ethyl acetate-methanol-water at volume ratios of (A) 8:2:5:5, (B) 7:3:5:5, (C) 6:4:5:5 and (D) 5:5:5:5 (v/v/v/v). Conditions: mobile phase: lower organic phase; flow rate: 2 ml/min; coil volume: 224 ml; revolution speed: 800 rpm; peak 1: impurity; peak 2: nevadensin.

the effect of different sample loading on the purity of nevadensin was shown in Table 4. It illustrated that with the sample loading increased from 2 g original sample to 4 g original sample, the purity of obtained nevadensin slowly decreased from above 98.0% to 97.2%; however, the retention of the stationary phase decreased from 51.2 to 43.1 and the separating degree reduced from 1.7 to 1.1. In general, the lower the retention of the stationary phase and the separating degree were, the worse the peak resolution. For the guarantee of the stability of the next step, 3 g original sample, with the most suitable retention of the stationary phase (48.0%) was selected as maximum of the sample load, and it was obviously that the sample load could be enlarged accordingly with the increase of the volume of coil column in preparative HSCCC.

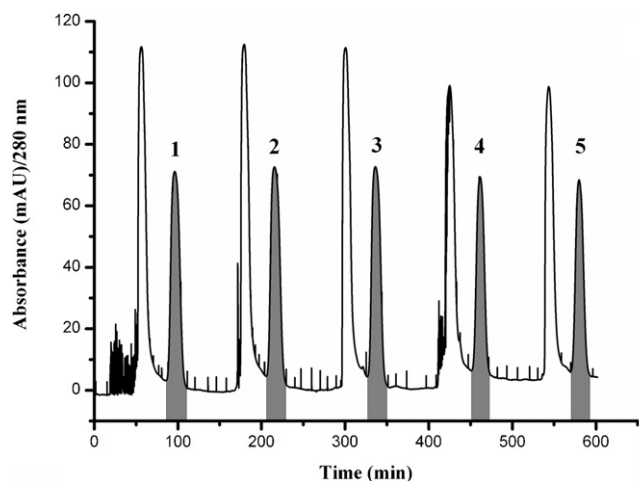
### 3.4. Application of current method

The critical experimental parameters of DMAE and HSCCC were optimized by above studies. It indicated that the mass of crude extracts obtained by DMAE was larger than the injecting sample mass of HSCCC and it was difficult to separate all crude extracts by HSCCC at a single separation process. The consecutive sample injections were selected to solve this problem as follow: when the previous separation process was completed, the crude extracts was directly injected into HSCCC column for next separation no need to reestablish hydrodynamic equilibrium of HSCCC. Therefore, this injection mode could save lots of separation time.

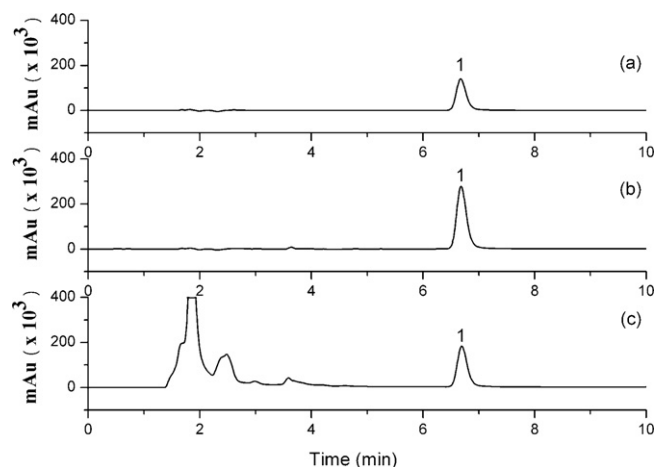
The on-line method was applied in isolation of nevadensin from *L. pauciflorus* Maxim. The extracts from 15 g original sample in three DMAE procedures were on-line concentrated and separated by HSCCC with five times sample injection under the optimal experiment conditions studied above. The HSCCC chromatograms were shown in Fig. 6. Result showed that the stationary phase retention

Table 4  
Effect of sample loading on the purity of obtained nevadensin.

Original sample (g)	Retention of stationary phase (%)	Separating degree	Purity of obtained nevadensin (%)
2	51.2	1.7	>98.0
3	48.0	1.4	>98.0
4	43.1	1.2	>98.0
5	43.1	1.1	97.2



**Fig. 6.** The HSCCC chromatogram of on-line isolation of nevadensin from *L. pauciflorus* Maxim. by coupling DMAE with HSCCC with five sample injections. Solvent system: hexane–ethyl acetate–methanol–water (7:3:5:5, v/v/v/v); stationary phase: upper phase; flow rate: 2 ml/min; revolution speed: 800 rpm; total sample amount: 15 g original sample; temperature: 25 °C; detection wavelength: 280 nm; peak (marked with 1, 2, 3, 4, 5): nevadensin.



**Fig. 7.** The HPLC chromatograms of 52 µg/ml nevadensin standard (a), collected fraction of HSCCC (b) and MAE extracted with 5 g original sample (c); peak 1: nevadensin.

just decreased 2.3% (from 50.5% to 48.2%) after five sample injections, which meant that the hydrodynamic equilibrium of HSCCC solvent system was stable. The total isolation mass of nevadensin was 13.0 mg, and the isolation yield of nevadensin was 0.87 mg/g. The average purity of nevadensin was higher than 98.0%, which met the demands of the isolation. Moreover, the entire isolation procedure, on-line coupled DMAE with HSCCC, was finished within less than 12 h, which was quiet short compared with other traditional isolation methods. All the results indicated that the combined method was effective and fast for high-throughput isolation of nevadensin from *L. pauciflorus* Maxim.

### 3.5. Identification of the separated peak

As shown in Fig. 7, HPLC analysis of all fractions from the HSCCC was compared with the standard sample and confirmed by the retention time and purity assay, the collected effluent fraction (marked with 1–5 in Fig. 6), according to the chromatographic profile was identified as nevadensin.

Moreover, the ESI-MS and  $^1\text{H}$  NMR data of the collected fraction was also given as follows: ESI-MS ( $m/z$ ): 244 [ $\text{M}^+$ ];  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 7.90 (2H, d,  $J=8.9$  Hz, H-3',5'), 7.05 (2H, d,  $J=8.9$  Hz, H-2',6'), 6.58 (1H, s, H-3), 4.05 (3H, s, 6-OCH<sub>3</sub>), 4.02 (3H, s, 8-OCH<sub>3</sub>), 3.90 (3H, s, 6-OCH<sub>3</sub>), which was accorded with reference [32], indicating the structural identification of nevadensin.

## 4. Conclusion

It was demonstrated that the crude extracts obtained from DMAE could be directly introduced into the HSCCC system for continuous isolation of nevadensin with five sample injections. 13.0 mg of nevadensin was obtained from 15 g sample of *L. pauciflorus* Maxim. with purity over 98.0% within less than 12 h, and the isolation yield was 0.87 mg/g. The presented method was simple, fast and efficient. Since the lack of standard compounds became the main limitation on the research and development of nature products, this method had good potential on the isolation of standards from nature products, especially on the quality control of TCMs.

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