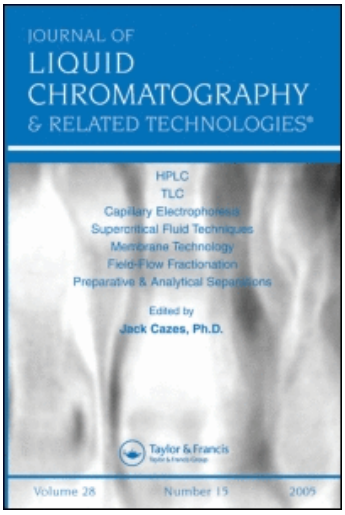

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Optimization of Conditions for Capillary Electrophoresis of Winged Euonymus by a Variable Dimension Expansion-Selection Method

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Abstract: A variable dimension expansion-selection method was proposed to rapidly optimize the separation of water extracts from winged euonymus by capillary electrophoresis (CE). A more efficient separation was achieved with the optimal conditions deduced from the method (50 mmol/L ammonium acetate buffer, pH 10.0). For comparison, a conventional univariate optimization approach with 19 experiments was applied. Its optimal conditions were the same as the optimal conditions deduced from our chemometric method with only 9 experiments. In addition, CE-MS (capillary electrophoresis-mass spectrometry) was applied to analyze the extracts with the optimal conditions for obtaining more fingerprinting information.

Keywords: Winged euonymus, Capillary electrophoresis, Variable dimension expansion-selection

INTRODUCTION

Herbal medicines have been applied in health care for a long time. After thousands of years of practice, about 700–800 kinds of crude herbal drugs are commonly used in China and other oriental countries.[1] Fingerprint analysis has been introduced and accepted by the World Health Organization (WHO) as a strategy for the assessment of herbal medicines. Recently, fingerprinting is also required by the Drug Administration Bureau of China to standardize usage of traditional Chinese medicines. The use of fingerprinting in

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herbs tends to focus on identifying and assessing the stability of the plants. High performance liquid chromatography (HPLC) fingerprint analysis has been reported as being used on some botanical medicines.[2] However, capillary electrophoresis (CE) is promising for the separation and fingerprinting analysis of herbal medicine because of its low sample consumption, rapid analysis, and high resolution. Online coupling of CE and MS (mass spectrometry) provides both the efficient separation of CE and the specificity of MS detection. Since the 1990s, CE-MS has developed significantly and has been proven to be a very useful tool in the analysis of proteins, drugs, etc.[1]

Winged euonymus (*Euonymus alatus (Thunb.) Sieb.*) is a traditional Chinese herbal medicine to regulate blood circulation, relieve pain, eliminate stagnant blood, and treat cancer. It can increase tolerance to oxygen deprivation and reduce blood sugar levels.[3]

Due to the variety of herbal components, the optimization of capillary electrophoresis conditions is time consuming. Electrophoresis separation is influenced by many experimental variables, such as voltage, temperature, injection time, and buffer composition including pH, addition of organic solvents, and modifiers. The most common strategy for optimizing the electrophoretic conditions involves a univariate approach in which each given experimental parameter is varied in a selected range, while keeping the remaining experimental conditions constant. Unfortunately, this method has two main drawbacks: (i) a complete study of all variables implies a large series of experiments, so it is costly and time consuming; (ii) interactions between two or more variables are neglected. If two or more variables interact, the conditions finally chosen might be far away from the optimum ones.[4]

Chemometric methods, such as uniform design[5,6] and simplex[7] have been employed for optimization in capillary electrophoresis. Many optimization methods (simplex, overlapping resolution maps, factorial designs, response surface methodology, neutral networks, etc.) in capillary electrophoresis and chromatography were reviewed[8] by Siouffi et al. The uniform design is to find a set of representative experimental points, which can scatter uniformly and regularly in the domain to be investigated. It can decrease the number of experiments significantly.[5] In addition, the variable dimension expansion-selection method, proposed by Goldberg and Zhang,[9–11] was employed to deal with the nonlinear relationship between trace elements in steel and the heating treatment of the steel.[12] This method was successfully applied to optimize CE conditions of extracts from ginseng.[13]

In this method, HCRF (hierarchical chromatographic response function)[6] was employed for electropherogram assessment. Uniform design was used for the experimental arrangement. The variable dimension expansion-selection method was applied to rapidly optimize the conditions of capillary electrophoresis of water extracts from winged euonymus, by obtaining the nonlinear relation between conditions and separation efficiency. For comparison, a conventional univariate optimization approach with 19 experiments was applied. Its optimal conditions were compared with our

chemometric method with 9 experiments. In addition, CE-MS was applied to analyze the extracts with the optimal conditions for obtaining more fingerprinting information.

THEORY AND METHODOLOGY

Assessment Function

Hierarchical chromatographic response function (HCRF) is a chromatographic assessment function proposed by Lu[14] to give an intuitive characterization of the separation quality of the chromatogram or electropherogram. The HCRF is defined as:

$$\text{HCRF} = Y = 10^6 n + 10^4 R_{\min} - (100 - t_m) \tag{1}$$

which n is peak number of electropherogram, $R_{\min}$ is minimal resolution of neighboring peaks, and t_m is migration time of the last peak (in minutes). The HCRF is defined as a dependent variable, Y, dependent on independent variables x_1, x_2, x_3, which are electrophoretic conditions.

Uniform Design

Three CE parameters, which are methanol concentration (x_1), pH (x_2), and concentration (x_3) of ammonium acetate buffer, were chosen for experimental arrangement of uniform design. Seven levels of each experimental parameter were taken. The experimental arrangement is shown in Table 1 (x_1, x_2, x_3). The experiments were shown as black points (•) in Figure 1, which scatter uniformly and regularly in the domain to be investigated.

Variable Dimension Expansion-Selection Method

Due to the nonlinear relationship between parameters and assessment function, the variable dimension expansion-selection method was processed. At first, three parameters (x_1, x_2, x_3) were expanded to 10 terms: x_1, x_2, x_3, x_1^2, x_2^2, x_3^2, x_1x_2, x_1x_3, x_2x_3, and $x_1x_2x_3$. Secondly, one combination of these terms forms one regression equation ($0 <$ term number $< m-1$, where m is the experiment number). For example, the combination of x_1, x_2^2, x_1x_3 forms the equation:

$$Y = b_0 + b_1x_1 + b_2x_2^2 + b_3x_1x_3 \tag{2}$$

where $b_k(k = 0 \ldots 3)$ is regression coefficients calculated by multivariable linear regression using the least squares method. Thirdly, all possible combinations of terms and their equations were investigated. Finally, the equation

Table 1. Arrangement of uniform design and corresponding results

No.	x_1 (%)	x_2 pH	x_3 (mmol/L)	n	$R_{\min}$	t_m (min)	Y (actual)	Y (calculated)
1	0	7.5	100	14	0.650	50.415	14006544	14003543
2	2	8.5	175	13	0.850	63.443	13008537	13009950
3	4	9.5	75	22	0.496	41.365	22005013	22006201
4	6	7.0	150	15	0.411	61.572	15004148	15005685
5	8	8.0	50	18	1.365	38.530	18013711	18017079
6	10	9.0	125	20	0.792	45.415	20007974	20001787
7	12	10.0	200	22	0.461	43.925	22004666	22006405

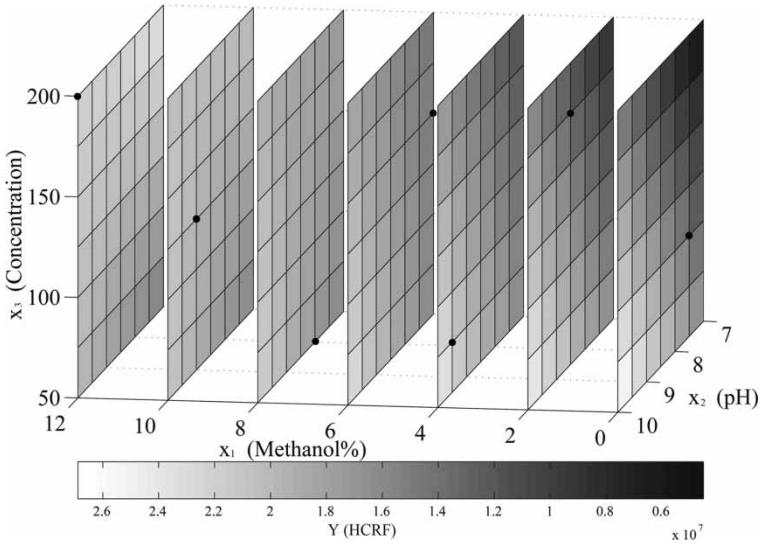

Figure 1. Three-dimension graph of the optimal equation and uniform designed points (●). 3 axes: methanol concentration (x_1), pH (x_2), and concentration (x_3, in mmol/L) of ammonium acetate buffer of capillary electrophoresis. Gray color represents the assessment function Y (HCRF, hierarchical chromatographic response function) of electropherograms. The points (●) represent the uniform designed experiments scattering uniformly in the domain.

with the least residual of error for Y was selected to be the optimal equation. This optimal equation was regarded as the most representative nonlinear relationship between Y (assessment of eletrophorogram) and parameters (electrophoretic conditions). The parameters which produce maximal Y by this equation were the optimal conditions.

EXPERIMENTAL

Reagents

Winged euonymus was purchased from northeast China. All other reagents were of analytical grade or higher. Double deionized water was used throughout. All buffers were filtered through 0.22 μm cellulose acetate membrane filters (Shanghai Xingya Resin Co. Ltd., Shanghai, China). Capillaries were purchased from Yongnian Optical Fiber Factory (Hebei, China).

Sample Preparation

Pulverized stem, 1.5 g, of winged euonymus was immersed in 20 mL water at room temperature for 24 h. It was then centrifuged at 20,000 rpm for 30 min.

The supernatant was filtrated, and dehydrated in vacuum at -54°C. The dry extract was stored at -20°C. It was diluted in 25 mL or 2.5 mL water before use in CE or CE-MS.

Apparatus

In chemometric optimization methods, experiments were carried out on a Beckman P/ACE 5000 capillary electrophoresis system using a 77 cm × 50 μm I.D. fused silica capillary, with a UV detector window at 70 cm. A constant applied voltage of 25 kV, a temperature of $20.0 \pm 0.1^{\circ}$C, and pressure injection for 30 s at 0.5 psi (1 psi = 6 894.76 Pa) were used.

In univariate optimization methods, experiments were carried out on a CE-212 capillary electrophoresis (Peking University) system using a 78 cm × 50 μm I.D. fused-silica capillary, with a UV detector window at 70 cm. A constant applied voltage of 19 kV, room temperature, and pressure injection for 30 s were used.

In CE-MS, experiments were carried out on an Agilent HP^{3D} CE-MSD Trap XCT capillary electrophoresis-mass spectrometry (electrospray ionization) system using an 87 cm × 50 μm I.D. fused-silica capillary. A constant applied voltage of 25 kV, a temperature of $20.0 \pm 0.1^{\circ}$C, and pressure injection for 30 s at 50 mBar (1 mBar = 100 Pa) were used. In MS systems, ions were detected in the negative mode. The pressure of the nebulizer was 10 psi. The flow rate of dry gas was 5 L/min. The dry temperature was 300°C. The scan range was 100–2000 m/z. The sheath liquid was a methanol solution of 0.1 mol/L ammonium acetate, and delivered at a flow rate of 15 μL/min.

In all CE above, UV detection was at 214 nm. The capillary was rinsed between runs with 0.2 mol/L sodium hydroxide for 2 min, water for 1 min, and running buffer for 2 min.

RESULTS AND DISCUSSION

Chemometric Method

The results are shown in Table 1 (n, $R_{\min}$, t_m, and Y). Average relative standard deviations (RSD) of the $R_{\min}$ and t_m were 9.3% and 5.8%, respectively. The optimal equation deduced from the variable dimension expansion-selection method was:

$$Y = 1.061 \times 10^7 - 8.017 \times 10^4 x_3 - 8.659 \times 10^4 x_1 x_2 + 2.043 \times 10^4 x_1 x_3 + 2.029 \times 10^5 x_2^2 - 1.313 \times 10^3 x_1 x_2 x_3 \quad (3)$$

A three-dimension graph of this equation was shown in Figure 1. Table 1 showed the calculated Y values of 7 experiments obtained from the

optimal equation. Similarity between actual and calculated Y was evidently shown.

Analyzing the equation or Figure 1, it was evident that the optimal set of parameters was: $x_1 = 0$, $x_2 = 10.0$, $x_3 = 50$. It produces the maximal Y (2.6×10^7). It represents CE conditions of 50 mmol/L ammonium acetate buffer (pH 10.0). Using the optimal conditions, a more efficient separation was obtained. Its peak number was 25, which was more than that of experiments above. The maximal Y calculated was $\sim 2.6 \times 10^7$, which implied 26 peaks according to equation (1). Due to similarity of actual and calculated maximal peak numbers (25 and 26), the optimal equation was validated as a representative nonlinear relationship between assessment function and experimental conditions.

The electropherograms of 7 uniform designed experiments and optimal separation is shown in Figure 2. It is evidently shown that the optimal separation was more efficient with most peaks and least analytical time.

Univariate Method

In the univariate method, each experimental parameter was varied in a selected range, while keeping the remaining parameters constant.

1. The effects of x_2 (pH of buffer) on the separation of the extracts were investigated over the pH range from 7.0 to 10.0, while keeping x_1 (methanol concentration = 0%) and x_3 (concentration of buffer = 200 mmol/L) constant. The result indicated that the best separation was achieved in buffer with pH 10.0.
2. The effects of x_3 (concentration of buffer) on the separation of the extracts were investigated over the range from 50 mmol/L to 200 mmol/L, while keeping x_1 (methanol concentration = 0%) and x_2 (pH of buffer = 10.0) constant. The result indicated that the best separation was achieved in buffer of 50 mmol/L.
3. The effects of x_1 (methanol concentration) on the separation of the extracts were investigated over the range from 0% to 20%, while keeping x_2 (pH of buffer = 10.0) and x_3 (concentration of buffer = 50 mmol/L) constant. The result indicated that the best separation was achieved in buffer without methanol.

The optimal conditions (50 mmol/L ammonium acetate buffer with pH 10.0) deduced from the univariate optimization method with 19 experiments were the same as the optimal conditions deduced from our chemometric method with 7 experiments.

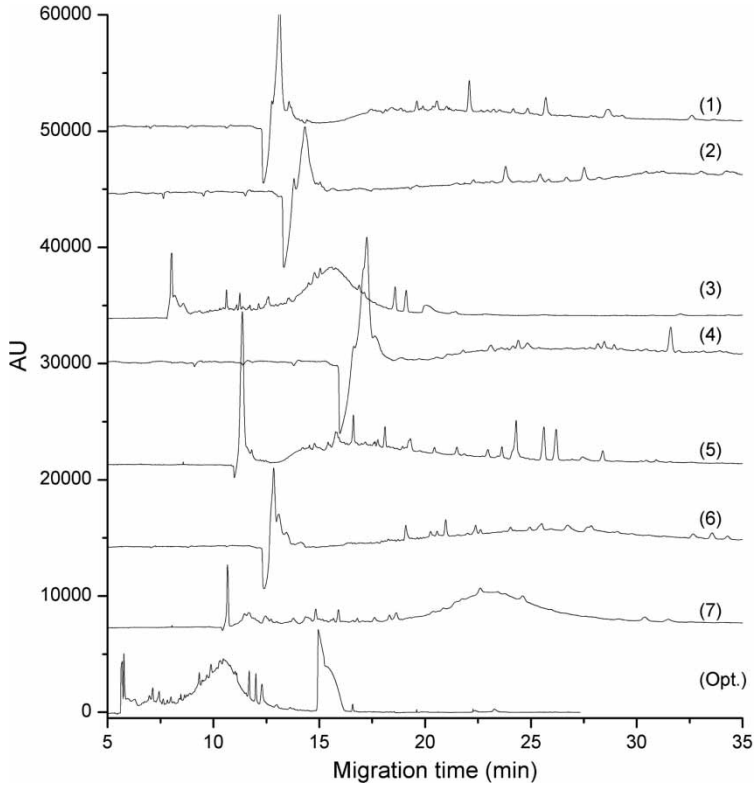

Figure 2. Electropherograms of uniform designed experiments (1–7) and optimal separation (Opt.) for extracts from winged euonymus with optimal conditions. Conditions: buffer, 50 mmol/L ammonium acetate solution (pH 10.0); applied voltage, 25 kV; temperature, $20.0 \pm 0.1^{\circ}$C; pressure injection for 30 s at 0.5 psi; detection wavelength at 214 nm.

Capillary Electrophoresis-Mass Spectrometry

Due to the scan limit of our CE-MS being 2000 m/z, only 4 main peaks are shown in the total ion current (TIC). The electropherogram and the main mass-to-charge (m/z) of peaks are shown in Figure 3. Further research is required on the interpretation of the result of CE-MS.

CONCLUSIONS

The variable dimension expansion-selection method was employed to optimize the separation of water extracts of winged euonymus by capillary electrophoresis. A more efficient separation was achieved with the optimal

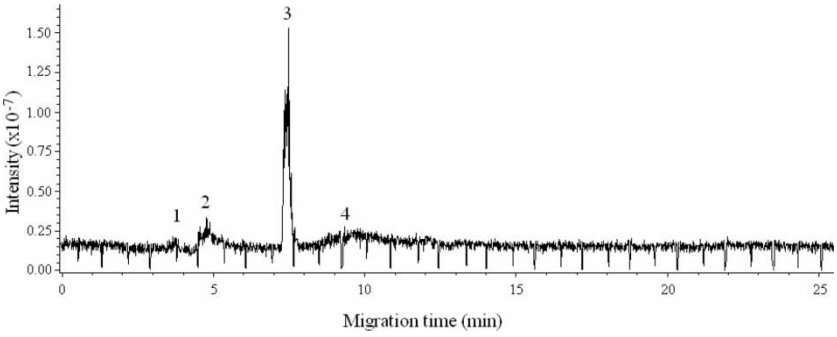

Figure 3. Electropherogram (CE-MS) and main m/z of peaks of extracts from winged euonymus. CE conditions: buffer, 50 mmol/L ammonium acetate solution (pH 10.0); applied voltage, 25 kV; temperature, 20.0 $\pm$ 0.1°C; pressure injection for 30 s at 50 mBar. MS conditions: detection mode, negative; pressure of nebulizer, 10 psi; flow rate of dry gas, 5 L/min; dry temperature 300°C; sheath liquid, methanol solution of 0.1 mol/L ammonium acetate; flow rate of sheath liquid, 15 μL/min. The main m/z of peaks: (1) 156.5; (2) 216.5; (3) 178.5 (4) 460.7.

conditions deduced from this method. For comparison, a conventional univariate optimization approach with 19 experiments was applied. Its optimal conditions were the same as the optimal conditions deduced from the chemometric method with 7 experiments. It is shown that this chemometric optimization method is rapid and efficient. The results show that the method described in this study is a useful tool to simplify optimization of conditions for analytical chemistry.

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