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Quick separation and determination of 6 lignans in Schisandra Chinensis (Turcz.) Baill and Schisand rasphenanthera Rehd. Et Wils using capillary electrophoresis

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ABSTRACT

To set up a method for quick determination of 6 lignans in Schisandra Chinensis using capillary electrophoresis. The paper discussed the influence of factors, such as buffer solution, additives, separation voltage, temperature and sample-loading conditions, on the separation and determination of lignans. The Schisandra chinensis sample solution can be analyzed under the optimal conditions of 10mmol/L NaH_2PO_4-Na_2HPO_4 buffer solution (35% acetonitrile, 37.5mmol/L SDS at pH 8) and separation voltage 28.0KV. The linear range is 25.5~1020, 25.75~1030, 12~480, 25.5~1020, 24.25~970 and 19.75~790 mg/L respectively; the detection limit is 0.5, 0.8, 1.0, 1.0, 1.0 and 1.5 mg/L. By comparing to the high performance liquid chromatography, the present method has remarkable advantages in the separation effect, column efficiency and analysis speed.

KEYWORDS

Capillary electrophoresis; Schisandra Chinensis; Lignans.

INTRODUCTION

Schisandra Chinensis is a traditional Chinese medicine. Its major plant elements include the ripped dried fruit of Schisandra chinensis (Turcz.) Baill. and Schisandra rasphenanthera Rehd. Et Wils. Both species are similar in shape and efficacy. The former is termed as Schisandra chinensis (Turcz.) Baill and the later is termed as chisandra rasphenanthera Rehd. Et Wils in China's Pharmacopoeia (Edition 2000). Schisandra chinensis has the efficacy of astringency, benefiting Qi and refreshing the saliva, tonifying the kidney and calming the heart [1]. It is typically believed that the contained lignans are the main physiologically-active components. The lignans have complicated components (more than 150 kinds of lignans have been found in Schisandraceae plants) with a lower content. The climate, place of origin and other factors pose significant influence on the composition and contents of lignans. However, the relevant studies have showed that 6 lignans: Schisandrin, Gomisin A, Schisantherin A, Deoxyschizandrin, Schisandrin B and Schisandrin C.

The capillary electrophoresis (CE) has the advantages of quick analysis speed, high column efficiency, low cost, free of population of capillary column and has unique advantages in the composition analysis of traditional Chinese medicine. No study on separation and determination of 6 lignans in Schisandra chinensis has reported using the CE approach. Štěrbová, et al has determined the contents of 4 lignans in Schisandra chinensis (Turcz.) Baill using micellar electrokinetic capillary electrophoresis (MEKC) approach, but the studies were lacked in sample treatment method, buffer salt choice, organic modifier and additives. The analysis time was long (above 18min) and there were fewer types of lignans (including 4 kinds of above 6 lignans). The present paper has investigated the influence of various factors on separation and determination of lignans systematically in MECK approach, reduced the analysis time (all components were at peak within 8.2min), created a MECK approach to quickly separate and determine 6 lignans in Schisandra chinensis, and created a new quicker, comprehensive, scientific method for evaluation and quality control of Schisandra chinensis.

EXPERIMENTAL PART

Instruments and reagents

Agilent CE meter is equipped with diode array detector (made by Agilent, USA); uncoated silica capillary (50μm * 56cm, at effective length 47.5cm, made by Hebei Yongnian Ruifeng Chromatographic Instrument Co., Ltd).

Schisandra chinensis (Turcz.) Baill was purchased from Anguo Medicine Market, Hebei; Dandong, Liaoning; Benxi, Liaoning; Wangqing, Jilin; Hulin, Heilongjiang. Schisandra rasphenanthera Rehd. Et Wils was purchased from local medicine market. The controls of Schisandrin, Gomisin A, Schisantherin A, Deoxyschizandrin, Schisandrin B and Schisandrin C were from NICPBP. Sodium dodecyl sulfate (SDS) was exported from Geneview by Beijing Dingguo Biotechnology Co., Ltd. The methanol, ethanol and acetonitrile were chromatographic reagents (Sinopharm Pharmaceutical Co., Ltd, Shanghai). Other reagents were analytic reagents and the experimental water was Millipore-prepared ultrapure water.

Preparation of Controls and Sample Solution

10.0mg Schisandrin, 10.0mg Gomisin A, 8.0mg Schisantherin A, 10.0mg Deoxyschizandrin, 10.0mg Schisandrin B and 8.0 Schisandrin C were measured and diluted to 1.00, 1.00, 0.80, 1.00, 1.00 and 0.80mg/ml in 10ml volumetric flasks after ultrasonic dissolving with methanol. The control storage solutions were kept at 4℃. 200μL control storage solutions were respectively pipetted, placed in 2ml centrifugal tubes, oscillated evenly in vortex way, and dried with nitrogen. The residue was resolved by adding 500μL buffer solution and ultrasonically treated for 20min to harvest the mixed control solution.

The dried Schisandra chinensis wad pulverized and sieved by 100 meshes. 10.0mg medicinal powder was weighed, added with 300ml methanol, ultrasonically extracted for 10min, shaken evenly and put aside for 10min, and then filtered. The filtrate was added with 150mL methanol and ultrasonically extracted for 10min. The residue was resolved with methanol, diluted to 100ml and stored at 4℃. 2ml solution was measured, and vacuum-evaporated. The residue was resolved with buffer solution and diluted to 2ml and filtered through 0.22μm film to harvest the sample solution.

Experimental methods

The buffer solution was 10mmol/L phosphate buffer solution (37.5mmol/L SDS, 35% acetonitrile, pH 8; 50mbar); pressure sampling at 50mbar for 3s; working voltage 28KV; detection wavelength 214nm; column temperature 25℃.

The capillaries were rinsed with 1.0 mol/L NaOH solution, 0.1mol/L NaOH solution, H_2O and buffer solution for 10min respectively; washed with buffer solutions before sample loading each time to ensure the better reproductibility.

RESULTS AND DISCUSSION

Buffer solution choice

In the experiment, the effects of HAC-NaAC, citric acid-sodium citrate, NaH_2PO_4-Na_2HPO_4 and boric acid-sodium borax buffer system were investigated on the separation effect of lignans. The results showed that the lignans were not at peak in HAC-NaAC and citric acid-sodium citrate buffer systems; but at peak in NaH_2PO_4-Na_2HPO_4 and boric acid-sodium borax buffer systems though at poor peak shape and separation degree. The preliminary optimization showed that better

separation effect could be derived in 20mmol/L NaH_2PO_4-Na_2HPO_4 buffer solution (30mmol/L SDS, 10% methanol) though in a longer analysis time (36min). We continued to optimize the separation conditions in order to obtain the best separation effect.

Buffer concentration choice

The result of investigating into the effects of different concentrations (5~25mmol/L) of NaH_2PO4-Na_2HPO_4 on the separation effect showed that the mobility time of each component increased as the concentration of buffer solution rose. By comprehensive consideration of mobility time, peak shape and sensitivity factors, etc, we chose 10.0mmol/L NaH_2PO4-Na_2HPO_4 as the buffer solution.

Effect of organic modifier

The organic modifier added in the buffer system can affect the mobility time, sensitivity, and separation effect and column efficiency. In the experiment, the effects of different amounts of added methanol, ethanol and acetonitrile were investigated on the separation effect. The results showed that the added methanol and ethanol in the buffer solution increased the mobility time and a certain amount of added acetonitrile reduced the mobility time apparently. In comparison of 20~40% (V/V) acetonitrile effect on separation, the results were showed as in Fig. 1. The mobility time and separation degree reduced as acetonitrile ratio rose. When the acetonitrile ratio was over 35%, the peaks of impurities that cannot be completed separated from the Gomisin A and Schisandrin B had the influence on the determination. By considering the mobility time and separation degree overall, we chose 35% acetonitrile as an organic modifier.

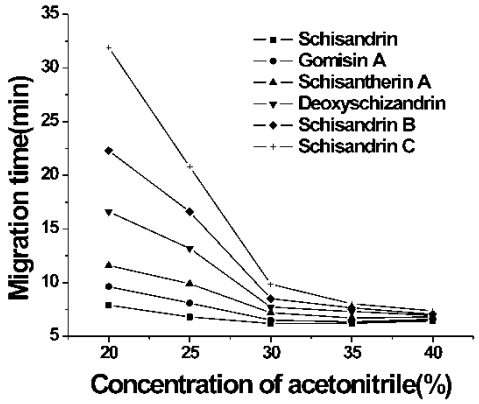

Figure 1 : Effect of varying the concentration of acetonitrile on mobilities of main lignans. Buffer: 30mM SDS and varying amount of acetonitrile in 10mM phosphate(pH 7.5). Applied voltage was 25 kV. Column temperature: 25.0 ℃. Detection: 214 nm.

Effects of Solution pH

By investigating into the effect of different buffer pH (7.0~9.0) on the separation effect as shown in Fig. 2, the mobility time reduced as the solution pH increased; the separation degree increased firstly (7.0~8.0) and then decreased gradually (8.0~9.0). By comprehensively taking into account of separation degree and mobility time, we chose pH8 buffer solution as buffer solution for electrophoresis.

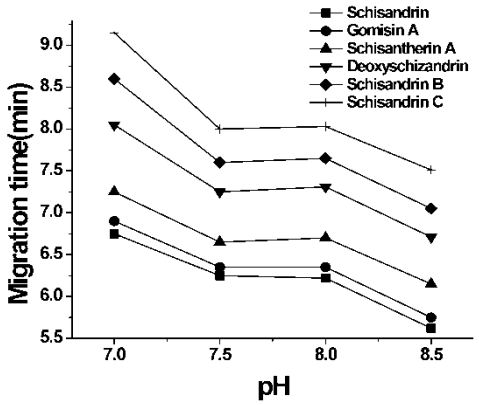

Figure 2 : Effect of varying the pH value of the buffer on mobilities of main lignans. Buffer: 30mM SDS and 35% v/v acetonitrile in 10mM phosphate. Applied voltage was 25 kV. Column temperature: 25.0 ℃. Detection: 214 nm.

Effect of surfactants

SDS is the most common anionic surfactant. In the experiment, the effect of 27.5~42.5mmol/L SDS was investigated on the separation degree as shown in Fig. 3. The mobility time and separation degree increased gradually whilst the column efficiency declined as the SDS concentration rose. By comprehensively taking into account of the separation degree and column efficiency, the optimal SDS concentration was 37.5mmol/L.

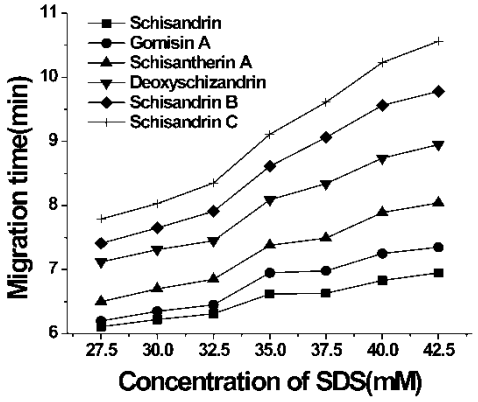

Figure 3 : Effect of varying the concentration of SDS on mobilities of main lignans. Buffer: 35% v/v acetonitrile and varying amount of SDS in 10mM phosphate(pH 8.0). Applied voltage was 25 kV. Column temperature: 25.0 ℃. Detection: 214 nm.

Choice of separation voltage, temperature and sample loading time

The samples were loaded and analyzed at 20~30 kV separation voltage. The results showed that the increasing voltage can improve column efficiency and speed up the analysis. However, if the voltage is too high, the separation degree will become worse along with the reduced analysis time and Joule heat effect. The optimal voltage is 28 kV.The influences of temperature was investigated on separation at 15～30℃. The results showed that the analysis was speeded up as the temperature rose. This is because the temperature rose, the solution viscosity is reduced and the mobility time is reduced. However, with a too high temperature, the analysis time is reduced whilst the samples diffusion is speeded up and the separation degree becomes worse resultantly. The optimal temperature is 25℃.

With pressured sampling, the amount of sample is subject to the sample loading pressure and time. If the amount of sample is far less, it is difficult to determine the samples; if the amount of sample is far more, the strip will be widened and the separation degree becomes worse. The optimal sample loading time is 3s at 50mbar.

METHODOLOGICAL VALIDATION

At the optimal conditions, the mixed control solutions were loaded 6 times repeatedly to determine the peak area and calculate RSD as shown in Table 1. A diagram is plotted with concentration (X: mg/L) of 6 lignans and peak area (Y). The regression equations, linear ranges and detection limits are shown in Table 1.

The control solutions at low, medium and high concentrations were added into the Schisandra chinensis (Turcz.) Baill produced in Dandong to determine the recovery rate as shown in Table 2.

Table 1 : Linearity, LOD, and RSD of the six lignan components

Substance name	Linear regression equation	Linearity (mg/L)	Detection limit (mg/L)	Reproducibility(% , n=6)
Schisandrin	Y = 0.8188X - 3.8212 r=0.9995	25.5-1020	0.5	1.08%
Gomisin A	Y = 0.4241X + 6.5037 r=0.9994	25.75-1030	0.8	1.19%
Schisantherin A	Y = 0.3816X - 3.3545 r=0.9993	12.0-480	1.0	1.79%
Deoxyschizandrin	Y = 0.3946x + 2.4609 r=0.9991	25.5-1020	1.0	1.83%
Schisandrin B	Y = 0.2706X - 0.7141 r=0.9993	24.25-970	1.0	1.80%
Schisandrin C	Y = 0.1474X + 4.9927 r=0.9983	19.75-790	1.5	1.98%

Table 2 : Recoveries of standard addition (n=6)

Substance name	Background(mg/L)	Added(mg/L)	Found(mg/L)	Recovery(%)	RSD(%, n=3)
Schisandrin	358.8	400	743.62	98.0	2.0
		200	543.71	97.3	2.3
		100	451.92	98.5	2.5
Gomisin A	213.95	400	622.55	101.4	2.3
		200	417.26	100.8	2.7
		100	321.8	102.5	3.1
Schisantherin A	102.5	200	294.94	97.5	3.3
		100	191.16	94.4	2.4
		50	144.88	95.0	2.7
Deoxyschizandrin	63.1	200	259.15	98.5	2.0
		100	159.19	97.6	2.8
		50	114.57	101.3	2.6
Schisandrin B	146.95	200	348.34	100.4	1.8
		100	252.14	102.1	2.2
		50	202.66	102.9	2.4
Schisandrin C	30.9	100	122.39	93.5	2.6
		50	77.91	96.3	2.9
		25	51.54	92.2	3.9

Sample analysis

Contents of 6 lignans in Schisandra chinensis samples from different sources (No. 1 Schisand rasphenanthera Rehd. et Wils was from Hunan; No. 2~6 Schisandra chinensis (Turcz.) Baill was from Benxi, Liaoning; Wangqing, Jilin; Shangzhi, Heilongjiang; Hulin, Heilongjiang) were respectively determined using MEKC HPLC methods created in the present paper(See Reference n). The electropherograms of Schisandra chinensis (Turcz.) Baill from Dandong, Liaoning and Schisand rasphenanthera Rehd. Et Wils from Hunan were shown in Fig. 4.

	Schisandrin		Gomisin A		Schisantherin A		Deoxyschizandrin		Schisandrin B		Schisandrin C	
	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC
1	1.058	1.143	0.623	0.652	4.521	4.997	5.821	5.523	0.496	0.434	0.761	0.800
2	7.176	6.937	4.279	4.031	2.050	2.163	1.262	1.374	2.939	3.188	0.618	0.694
3	4.730	4.532	3.550	3.375	0.993	1.094	0.811	0.856	3.398	3.611	0.536	0.562
4	6.142	5.999	3.436	3.272	1.892	1.993	1.206	1.327	3.744	3.952	1.275	1.390
5	6.255	6.041	4.289	4.127	2.254	2.482	1.045	1.138	3.602	3.828	0.593	0.621
6	4.851	4.665	4.838	4.620	1.409	1.556	0.887	0.953	3.553	3.787	0.838	0.918

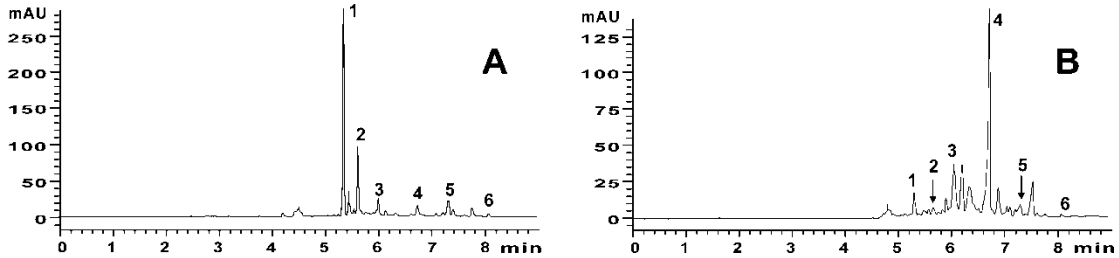

Figure 4 : Electropherograms of (A) *Schisandra chinensis* and (B) *Schisandra sphenanthera* Peak identification: 1, Schisandrin; 2, Gomisin A; 3, Schisantherin A; 4, Deoxyschizandrin; 5, Schisandrin B; 6, Schisandrin C. MEKC conditions: background electrolyte, 37.5mM SDS and 35% v/v acetonitrile in 10mM phosphate buffer (pH 8.0). Applied voltage was 28 kV. Column temperature: 25.0 ℃. Detection: 214 nm.

CONCLUSION

In the present paper, 6 lignans: Schisandrin, Gomisin A, Schisantherin A, Deoxyschizandrin, Schisandrin B and Schisandrin C were simultaneously separated and determined. With systematic studies on the influence of factors on separation and determination, the optimal electrophoresis condition was determined so that the analysis speed is increased in

a more drastic magnitude than existing methods (8.2min in the present method, above 18min in reported MEKC method and above 30min in HPLC method). Results derived from 10 Schisandra chinensis samples from different places using the present method were similar to what was obtained using HPLC method. The present method created a new quicker, comprehensive, scientific method for evaluation and quality control of Schisandra chinensis.

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