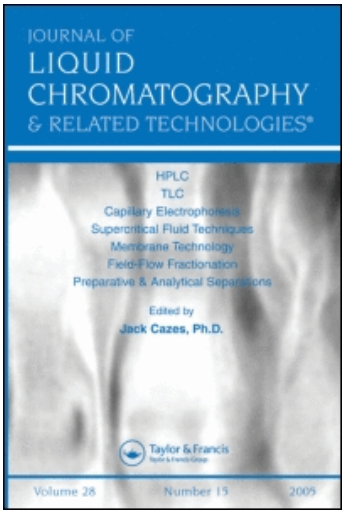

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Separation of Tanshinone I, Tanshinone IIA, and Cryptotanshinone from *Salvia miltiorrhiza Bunge* by Normal Phase HPLC

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Abstract: A normal phase high performance liquid chromatographic method was established for the simultaneous determination of tanshinones in *Salvia miltiorrhiza Bunge*. Tanshinones, including cryptotanshinone, tanshinone I, and tanshinone IIA were successfully separated on a silica column using the mixture of n-hexane and dichloromethane (96/4, v/v) as mobile phase. The results of the quantitative experiments proved that methanol is the best solvent in the five common solvents. Linear equations revealed good linear relationship between the peak areas of tanshinones and their concentrations. The effect of the dipping time and ultrasonic time were measured, the trend lines dependent on the linear equations were drawn, and the ultrasonic condition was better than the dipping condition.

Keywords: Cryptotanshinone, Normal phase high performance liquid chromatographic method (NP-HPLC), Tanshinone I, Tanshinone IIA

INTRODUCTION

Traditional Chinese medicinal herbs have been widely used for over 2000 years. Most traditional Chinese medicinal preparations are composed of complex constituents, and proper methods are required for their quality

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control. Many high performance liquid chromatographic (HPLC)[1–4] and capillary electrophoresis methods have been developed for the determination of marker constituents in traditional Chinese medicinal preparations.[5,6]

Salvia miltiorrhiza Bunge, Dan-Shen in Chinese, is a well known traditional Chinese medicinal herb used for treatment of various kinds of diseases, especially for coronary disorders.[7] The major active constituents of this herb are tanshinones, including tanshinone I, tanshinone IIA, and cryptotanshinone. It was reported that tanshinones can dilate coronary arteries, increase coronary flow, modulate mutagenic activity, and protect the myocardium against ischaemia. Pharmacological tests revealed that all these components have an anticancer effect, and among these tanshinones, tanshinone IIA is most effective and has been used as a quality controller for some medicines.[8] They also have some activity as a broad spectrum bactericide. The chemical structures of these tanshinones are given in Figure 1.

Recently, the researches on the separation and purification of tanshinones using the conventional methods such as the reversed phase high performance liquid chromatographic method (RP-HPLC) have gotten good results and the separation conditions were also well determined.[1–4] But the separation of tanshinones depending on normal phase high performance liquid chromatography (NP-HPLC) is still a new area,

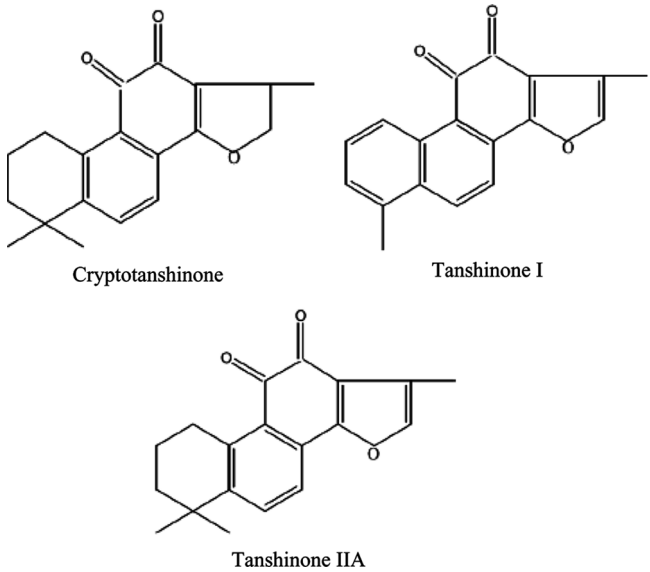

Figure 1. The structures of the tanshinones.

the feasibility and the appropriate separation conditions of the model are all the problems that remained to be solved.

The present paper describes the separation of tanshinones from a crude extract of *Salvia miltiorrhiza Bunge* by NP-HPLC; a series of experiments were done to find the appropriate mobile phase, then the linear equations of the tanshinones using the standard solutions were calculated based on the linear equations to get the other optimal separation conditions: solvent, dipping time, and ultrasonic time. Altogether, the qualitative analysis and quantitative analysis were done to get the appropriate separation conditions.

EXPERIMENTAL

Materials and Reagents

Authentic standards of cryptotanshinone, tanshinone I, and tanshinone IIA were purchased from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. All other reagents were of analytical grade and purchased from Duksan Pure Chemicals Co., Ltd., Ansansi, Korea. The water used was doubly distilled. The Dan-Shen powder was purchased from a local market in Korea.

Apparatus and Chromatographic Conditions

The HPLC system was equipped with Waters 600 S solvent delivery system including 616 solvent delivery pump, 600 S controller, the 2487 UV dual channel detector, and an injector (0.02 mL sample loop) of rheodyne (Waters, Milford, MA, USA). The data acquisition system was Millenium32 (Waters), installed in a Sumsung PC. The wavelength was fixed at 254 nm. Separation was accomplished on a silica column (4.6×250 mm, 5 μm). The flow rate was set at 0.5 mL/min. and the column temperature was maintained at room temperature.

Preparation of Standard Solutions

Stock standard solutions of cryptotanshinone, tanshinone IIA, and tanshinone I were prepared in methanol. Working standard solutions containing each of the three compounds were prepared by diluting the stock solutions with methanol to proper volumes. The standard stock solutions and working solutions were all prepared in dark brown calibrated flasks and stored at 4°C until analysis.

Preparation of Dan-Shen Sample Solutions

Dan-Shen powder (1.0 g) was added into a glass bottle (100 mL capacity) with 50 mL of solvent at room temperature. Then, the mixtures were stirred or ultrasonicated at different times according to the concrete requirements in each operation. After that, an aliquot of the solution was filtered through a 0.20 μm disposable syringe filter. Finally, 5 μL of the filtrate was injected into the HPLC system for analysis.

RESULTS AND DISCUSSION

Selection of the Mobile Phase

The former studies on the separation of cryptotanshinone, tanshinone I, and tanshinone IIA used NP-HPLC are few, so we have to try each one. At first, we used pure n-hexane, the most common mobile phase for the NP-HPLC, but it just showed a wider peak in the chromatography, which cannot be separated. Then dichloromethane was added into n-hexane as the additive; the volume rate of n-hexane/dichloromethane ranged from 95/5 to 50/50 (v/v) but, also, the tanshinones could be clearly separated. We analyzed that maybe the polarity of dichloromethane was not sufficient, so we changed to 2-propanol with larger polarity.

The result proved that when the mobile phase was added in 2-propanol at low volume ratio, the cryptotanshinone, tanshinone I, and tanshinone IIA can be adequately separated. All of the volume ratios of n-hexane to 2-propanol ranging from 98/2 to 95/5 have good separation effects; considering the appropriate retention time (between 10 min and 30 min), we chose the volume ratio of 96/4 as the optimal one to prepare the mobile phase. The chromatogram of the Dan-Shen sample solution is shown in Figure 2.

Linear Regression Equations

The establishment of mobile phase is the foundation of qualitative analysis, and the linear regression equation is the foundation of quantitative experiments, including the selection of the solvent and the establishment of the optimal dipping time and the ultrasonic time. All calibration graphs were plotted based on linear regression analysis of the integrated peak areas (Y) versus concentrations (X) of the tanshinones in the standard solution at six different concentrations (each concentration injected

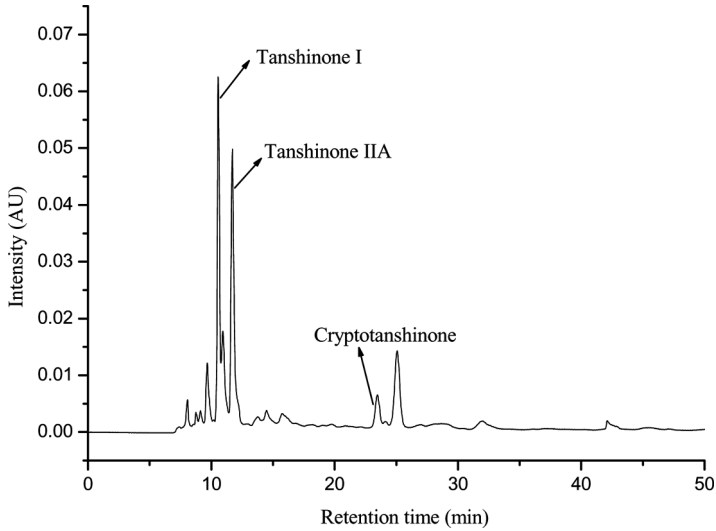

Figure 2. The chromatogram of the Dan-Shen sample solution.

three times). The linear regression equations, correlation coefficients, and linear ranges for the analysis of the tanshinones are shown in Table 1.

In it, all of the correlation coefficients were larger than 0.96, which revealed good linear relationship between the peak areas of tanshinones and their concentrations, so they can be used in the following quantitative experiments.

Selection of the Solvent

In the former steps, we used methanol as the solvent of the standard samples. In order to validate whether methanol is the best solvent for tanshinones or not, we prepared five common solvents: methanol, ethanol, ethyl acetate, chloroform, and water; 1 g Dan-Shen powder

Table 1. The linear equations of cryptotanshinone, tanshinone I and tanshinone IIA

Compounds	Linear equation	Linear range (μg/mL)	r^2
Cryptotanshinone	$Y = 865044X – 1000000$	$0.139 \sim 83.33$	0.9635
Tanshinone I	$Y = 167972X – 258363$	$0.139 \sim 83.33$	0.9624
Tanshinone IIA	$Y = 766652X – 1000000$	$0.139 \sim 83.33$	0.9603

X denotes concentration (μg/mL) of the tanshinones, Y denotes peak area.

Table 2. The extraction concentrations of tanshinones in different solvents

Solvent	Concentration		
	Tanshinone IIA (μg/g)	Tanshinone I (μg/g)	Cryptotanshinone (μg/g)
Methanol	0.0835	0.115	0.0604
Ethanol	0.0793	0.101	0.0581
Ethyl Acetate	0.0830	0.0828	0.0583
Chloroform	0.0655	0.0792	0.0580
Water	*	*	*

The liquid/solid ratio is 50:1, "*"not detected.

was dipped in solvents for 20 hr, and then the results compared to select the best one.

The concentrations of the tanshinones in different solvents were calculated using the linear equation. The results are shown in Table 2.

According to Table 2, the highest extracting concentrations of tanshinones were in methanol, so we used methanol as the solvent for the extraction. It is well known that in traditional Chinese medicine, medicinal herbs are usually dipped in water and then heated to get the medicinal compositions, but in this experiment, all of cryptotanshinone, tanshinone I, and tanshinone IIA were not detected, it might be because of the tanshinones could not be dissolved in water.

Establishing the Optimal Dipping Time

In order to obtain the optimal dipping time, the extracting solution in different dipping times: 20 min, 40 min, 1 hr, 2 hr, and 4 hr were prepared. The trend concentration lines of the tanshinones depending on the dipping time was shown in Figure 3.

According to the trend line in the Figure 3, before 2 hr all of the concentrations of the tanshinones increase linearly, but after 2 hr they remain with almost no changes. So, the optimal dipping time is 2 hr, and the concentration magnitude of the tanshinones was: Tanshinone I > Tanshinone IIA > cryptotanshinone.

Establishing the Optimal Ultrasonic Time

Generally, ultrasonication has a great effect on the homogeneity of the solution, thus increasing the accuracy of the experiment date.

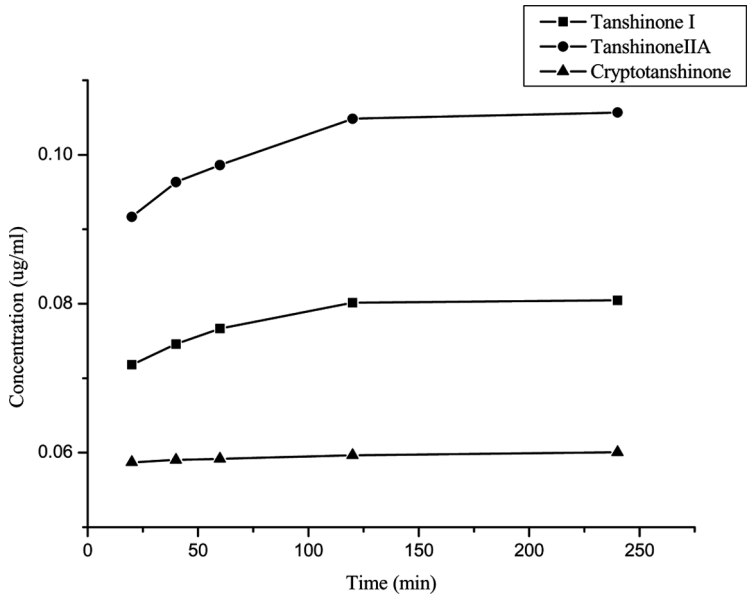

Figure 3. The concentrations of tanshinones at different dipping times.

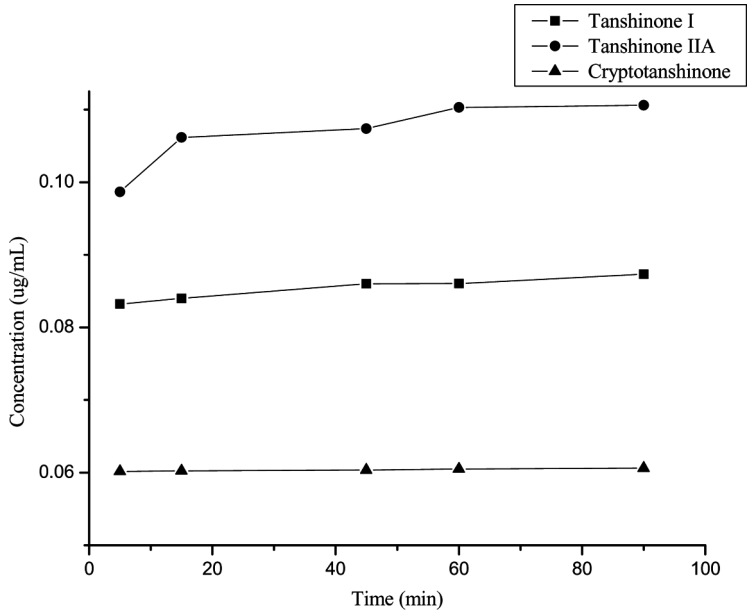

Figure 4. The concentrations of tanshinones at different ultrasonication times.

Ultrasonication also effects the solubility of cryptotanshinone, tanshinone IIA, and tanshinone I. In order to evaluate the effect of ultrasonication time to the concentration of the tanshinones, and get the optimal ultrasonication time, the extracting Dan-Shen solutions in different ultrasonication time were prepared, the ultrasonication time was 5 min, 15 min, 45 min, 1 hr, and 1.5 hr (power, 45 watt), respectively. The concentrations also were calculated by the linear equation.

According to Figure 4, the slopes of the concentration/ultrasonic time line are low, especially for the cryptotanshinone, there is nearly no change in the concentration as the time increases. However, comparing the results of the ultrasonication and dipping methods, it was found that the amounts extracted via the ultrasonication method were higher. Thus, it was determined that the dipping method is not appropriate for this approach.

CONCLUSIONS

The establishment of the mobile phase reflected that it's feasible to separate cryptotanshinone, tanshinone I, and tanshinone IIA from Dan-Shen by NP-HPLC. Through the quantitative experiments and the analysis of the control experiments, the optimal separation condition was obtained: the mobile phase was n-Hexane/2-propanol (96/4, v/v), the liquid/solid ratio was 50 mL/g, the solvent for the extracting solution was methanol, the dipping time was 2 hr, the ultrasonication time was 45 min.

REFERENCES

1. Shi, Z.H.; He, J.T.; Yao, T.T; Chang, W.B.; Zhao, M.P. Simultaneous determination of cryptotanshinone, tanshinone I and tanshinone IIA in traditional Chinese medicinal preparations containing *Radix salvia miltiorrhiza* by HPLC. J. Pharm. Biomed. Anal. **2005**, *37*, 481–486.
2. Xue, M.; Cui, Y.; Wang, H.Q.; Hu, Z.Y.; Zhang, B. Reversed-phase liquid chromatographic determination of cryptotanshinone and its active metabolite in pig plasma and urine. J. Pharm. Biomed. Anal. **1999**, *21*, 207–213.
3. Mao, S.J.; Hou, S.X.; Liang, Z.; Bi, Y.Q.; Wu, Y.; Li, H.; Jin, H. Ion-pair reversed-phase HPLC: Assay validation of sodium tanshinone IIA sulfonate in mouse plasma. J. Chromatogr. B. **2006**, *831*, 163–168.
4. Banasri, H.; Madhushree, D.S.; Utpal, S. Separation methods of quinonoid constituents of plants used in Oriental traditional medicines. J. Chromatogr. B. **2004**, *812*, 259–275.
5. Takei, H.; Nakauchi, K.; Yoshizaki, F. Analysis of swertiamarin in swertia herb and preparations containing this crude drug by capillary electrophoresis. Anal. Sci. **2001**, *17*, 885–888.

6. Yang, J.J.; Long, H.; Liu, H.W.; Huang, A.J.; Sun, Y.L. Analysis of tetrandrine and fangchinoline in traditional Chinese medicines by capillary electrophoresis. J. Chromatogr. A. **1998**, *811*, 274–279.
7. Luo, H.W.; Wu, B.J.; Wu, M.Y.; Yong, Z.G.; Niwa, M.; Hirata, Y. Pigments from *Salvia miltiorrhiza*. Phytochemistry. **1985**, *24*, 815–817.
8. Tian, G.L.; Zhang, Y.B.; Zhang, T.Y.; Yang, F.Q.; Yoichiro, I. Separation of tanshinones from *Salvia miltiorrhiza Bunge* by high-speed counter-current chromatography using stepwise elution. J. Chromatogr. A. **2000**, *904*, 107–111.

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