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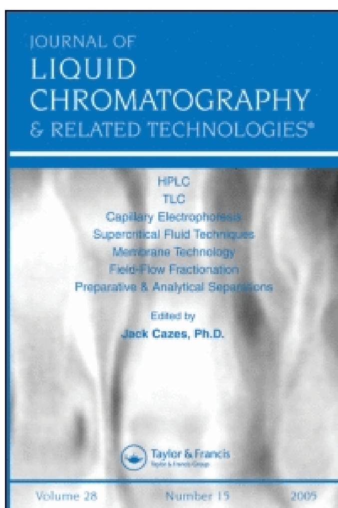

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Development and Validation of a Method for the Determination of Tanshinones in Supercritical Fluid Extraction Products by HPLC

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Abstract: Supercritical fluid extraction has been merging as a promising alternative technique for the extraction of the lipid soluble compounds from natural resources for the nutrient food. However, to evaluate SFE process and its products, it is usually only by measuring one of the main constituents or the extract yield, which sometimes would be thought as defective and unreasonable. In this article, we presented a multi-active component analysis method to evaluate the SFE of *Salvia miltiorrhiza.* A reverse-phase HPLC method for the simultaneous analysis of the four tanshinones (dihydrotanshinone I, cryptotanshinone, tanshinone I and tanshinone II_A) from the SFE products was developed with a phase composition as water/acetonitrile. The gradient elution of the mobile phase was 60% (acetonitrile) in 0–17 min, 60–80% in 17–25 min, and 80% in 25–30 min. By this comprehensive evaluation method, the SFE process of *Salvia miltiorrhiza* was investigated and variables involved in the procedure such as pressure, temperature, and extraction time were optimized.

Keywords: Supercritical fluid extraction, Tanshinone, *Salvia miltiorrhiza*, Chinese herb, Food analysis

INTRODUCTION

Recently there has been an increasing interest in research on nutrient food components from Chinese herbs such as the *Danshen*, the dried root of

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Salvia miltiorrhiza, because of its possible linkage to health benefits, e.g., reduction in coronary heart disease, cardiovascular and cerebrovascular diseases.[1,2] There are more and more patents about *Salvia miltiorrhiza* nutrient food, such as *Salvia miltiorrhiza* tea, being applied for.[3–5] However, as to the application of the extract of *Danshen*, according to the Chinese pharmacopoeia,[6] only the tanshinone II_A [$C_{19}H_{18}O_3$, CASRN 568-72-9] is particularly recorded for the lipid soluble tanshinones. This would not be comprehensively representative of the tanshinones' biological activities. In recent years, dihydrotanshinone I [$C_{18}H_{14}O_3$, CASRN20958-19-4], cryptotanshinone [$C_{19}H_{20}O_3$, CASRN 17545-07-2], tanshinone I [$C_{18}H_{12}O_3$, CASRN568-73-0], and tanshinone II_A (Figure 1), have been identified as the major active lipid soluble constituents in *Salvia miltiorrhiza*.[7,8] Pharmacological experiments demonstrated that dihydrotanshinone I possesses the function of the cell growth arrest and apoptosis induction in cancer cells, cryptotanshinone is usually used against inflammation, tanshinone I for therapy of angina pectoris, tanshinone II_A for improving blood circulation.[1,9]

Supercritical fluid extraction (SFE) has been merging as a powerful technique for the extraction of the lipid soluble compounds from natural resources for nutrient foods. Since it has the remarkable advantages, such as less consumption of hostile organic solvents, reduction of the extraction time, avoidance of degradation of thermally labile compounds, and production of cleaner extracts,[10] SFE has a significant development in the extraction of *Salvia miltiorrhiza*, especially in China, and many papers[11–14] and

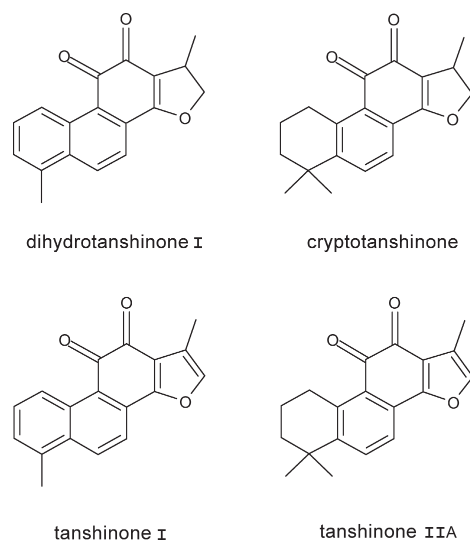

Figure 1. Molecular structures of four tanshinones.

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patents[15–17] have been published. Among them, we noticed that the determination of the major constituents in the SFE products was relatively monotone and ambiguous. Most of them only focused on the content and recovery of tanshinone II_A, while other tanshinones, such as dihydrotanshinone I, cryptotanshinone, and tanshinone I were often overlooked. However, these tanshinones, especially dihydrotanshinone I and cryptotanshinone, may cause serious toxic effects in humans and their daily intake should be seriously controlled.[18] So, establishing a valid analysis method for monitoring the active compounds in these products will be meaningful for the application of SFE in the food production areas. Further, the method using the instrumentation is widely available in quality control laboratories.

Up to now, many methods have been developed for the determination of tanshinones or other bioactive constituents in *Salvia miltiorrhiza*,[19–23] including the analysis of dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone II_A. For instance, some methods for simultaneously determining the four tanshinones in the traditional chinese medicines preparation were reported recently,[23–25] but a relatively higher standard of chromatography conditions was required during the analysis. Moreover, the mobile phase used was a mixture of methanol, tetrahydrofuran, water and glacial acetic acid, which was rather complex and tedious. Also, adding the glacial acetic acid is harmful to the column and requires the column to be more antacid. Thus, it is necessary to find a convenient and cheap method for simultaneous analysis of the four tanshinones as an improved quality control analysis for SFE product evaluation. The aim of the presented study was to develop and validate a new HPLC for the simultaneous determination of the four tanshinone compounds in SFE extracts with the analysis conditions easier and the procedure more convenient and economical.

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EXPERIMENTAL

Materials and Reagents

Dried root of *Salvia miltiorrhiza* was purchased from Tongrentang Medicine Co. Ltd. (Peking, China). Material size consisted of pieces, the diameter and thickness of which was about 3 and 3–5 mm, respectively.

HPLC grade acetonitrile and methanol were from Merck (Darmstadt, Germany). Ultrapure water was prepared by using a Milli-Q academic water purification system (Milford, MA, USA). Reference standards of dihydrotanshinone I (CAS 20958-19-4), cryptotanshinone (CAS 17545-07-2), tanshinone I (CAS 568-73-0), and tanshinone II_A (CAS 568-72-9) were purchased from Zhejiang Chemical Reagent Plant, China. CO_2, with the purity of 99.5%, was kindly supplied by Hangzhou Electrochemical-Gas Factory (Hangzhou, China). The other chemicals, such as ethanol were used as received.

Supercritical Fluid Extraction (SFE)

The experiment was carried out on a HA220-50-06 extraction system (Hua An SFE Company, Jiangsu, China) equipped with a 5 L volume extractor, two 1 L separators, 100 DX syringe pump, and a syphonated carbon dioxide (CO_2) cylinder that was pressurized up to work pressure. The extraction pressure of the system was adjusted by a pressure regulator and its temperature was controlled by a thermostatic water bath. A mass of 1.5 kg of the plant material was loaded into the extractor. After 1 h of static extraction (no CO_2 flow), the sample was subjected to dynamic extraction by supercritical-CO_2. Also, 500 mL of ethanol as modifier was subjected to the extractor by another pump. The extract was collected from the bottom of the separator and evaporated to dryness at 60°C in vacuum condition.

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HPLC Analysis

The HPLC system consisted of an Agilent 1100 series HPLC system (Agilent Company, USA) equipped with a quaternary pump, online degasser, column heater, autosampler, and a UV detector. Data collection and analysis were performed using ChemStation software (Agilent Technologies, Wilmington, DE). Separation was achieved on a Lichrospher C_{18} column (250 mm × 4.6 mm i.d., 5.0 μm particle size) from Hanbang Science & Technology (Jiangsu, China) coupled with an Agilent C_{18} precolumn (4 mm × 5 mm). The column temperature was maintained at 30°C and the injection volume of the sample was 10 μL.

Preparation of Standard Solution

Primary standard stock solutions for the investigated compounds, i.e., dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone II_A were prepared in methanol with concentrations of 0.1 mg/mL, respectively. This solution was stored away from light at 4°C until used for analysis.

Preparation of Sample Solution

The product of SFE (about 2 mg) was accurately weighed, put into a clean dry 25 mL volumetric flask, and dissolved with methanol. The obtained solution was then centrifuged at 10,000 rpm for 5 min. After filtering through a 0.45 μm membrane filter, the supernatant was injected into the HPLC system for analysis.

Validation of the Method

The analytical method developed was tested as regards linearity, limits of detection and quantification, precision, repeatability, stability, and recovery.

The quantification of the chromatogram was performed using the peak areas of four investigated compounds. Six standard solutions were prepared and subjected to triplicate analysis by HPLC. The peak area versus concentration was plotted in the concentration range from 1 to 16 μg/mL.

The standard solution containing the four reference compounds was further diluted to provide appropriate concentrations for the gain of the limits of detection (LOD) and quantitation (LOQ). LOD and LOQ were calculated in accordance with at a signal-to-noise (S/N) of 3 and 10, respectively.

The intra- and inter-assay precision was determined by a standard mixture solution of the four tanshinones under the selected chromatography conditions, six times in a day for intra-assay precision and once a day on three consecutive days for inter-assay precision. The relative standard deviation (RSD) was taken as a measure of the intra- and inter-day precisions. To evaluate the repeatability, five different working solutions prepared from the same product of SFE were analyzed. For the stability test, the same sample solution was analyzed at different times within 72 h at room temperature. And each solution was analyzed in triplicate under the best chromatographic condition.

A standard addition test was performed to determine recoveries of the four tanshinones. In this assay, the standard solutions with different concentration levels were prepared and added to the samples with known content. The resultant samples were analyzed using the developed HPLC method mentioned above. The experiments were repeated three times for each level. The ratio of measured and added amounts was used to calculate the recovery.

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RESULTS AND DISCUSSION

Extraction Method Development

In order to obtain quantitative extraction, variables involved in the procedure such as pressure, temperature, and extraction time were optimized. Each factor was analyzed for three levels, respectively. The mean values of the three levels of each factor reveal how the extraction yield changes when the level of that factor is changed. The influence of pressure on the efficiency of extraction was investigated, in which powdered samples were extracted under the pressure of 20, 30, and 40 MPa, respectively. The results, as Figure 2 shows, suggested that the highest amount of tanshinones were obtained under the pressure of 40 MPa. Then three temperatures (40, 50, and 60°C) were evaluated to optimize the extraction process. The experiments show that the tanshinones yields were improved when the extraction temperature increased from 40 to 50°C, but, increasing the temperature from 50 to 60°C decreased the

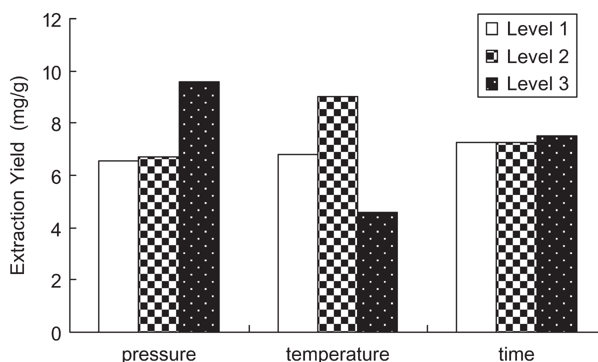

Figure 2. The extraction yields of *Salvia miltiorrhiza* under different conditions.

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efficiency of the tanshinones and the highest extraction yield was obtained when temperature was at 50°C. The experiments also indicated that the most of the products came out in 1 h, and the extraction yields of tanshinones didn't increase significantly with the extraction time extended to 2 or 3 h. Thus, the best conditions obtained by test for the extraction of tanshinones were: extraction pressure: 40 MPa; temperature: 50°C; dynamic extraction time: 1 h (Figure 2).

HPLC Separation Optimization

The selection of the HPLC conditions was guided by the requirement for obtaining chromatograms with better resolution of adjacent peaks within a short time. A wavelength of 254 nm or 270 nm is ordinarily used for the detection of tanshinones in *Salvia miltiorrhiza* preparation samples. At the wavelength 254 and 270 nm, each tanshinone showed strong absorption. According to the Chinese Pharmacopoeia, the determination of the tanshinone II_A is performed in the wavelength of 270 nm. In addition, since the peak areas of the four tanshinones under the wavelength of 270 nm have higher response values than 254 nm, we selected 270 nm as the appropriate wavelength for the detection of tanshinones.

Then we optimized the mobile phase composition using an isocratic elution system with flow rate of 1 mL/min. In most studies published to date, the methanol/tetrahydrofuran/water/glacial acetic acid system was used for tanshinone analysis (Shi et al., 2005; Zhang et al., 2005). However, using that system, the procedure of preparing for the mobile phase was rather complex, tedious, and highly consumptive. Therefore, we tried to achieve a good separation by using the water/acetonitrile system. Though the water/acetonitrile system didn't yield markedly better separation between the cryptotanshinone and tanshinone I, their peak purities were adequate for quantification. Then, we tried to use the gradient elution method and used the mobile phase composition as water/acetonitrile (35:65), with the result that the peaks of cryptotanshinone and tanshinone I

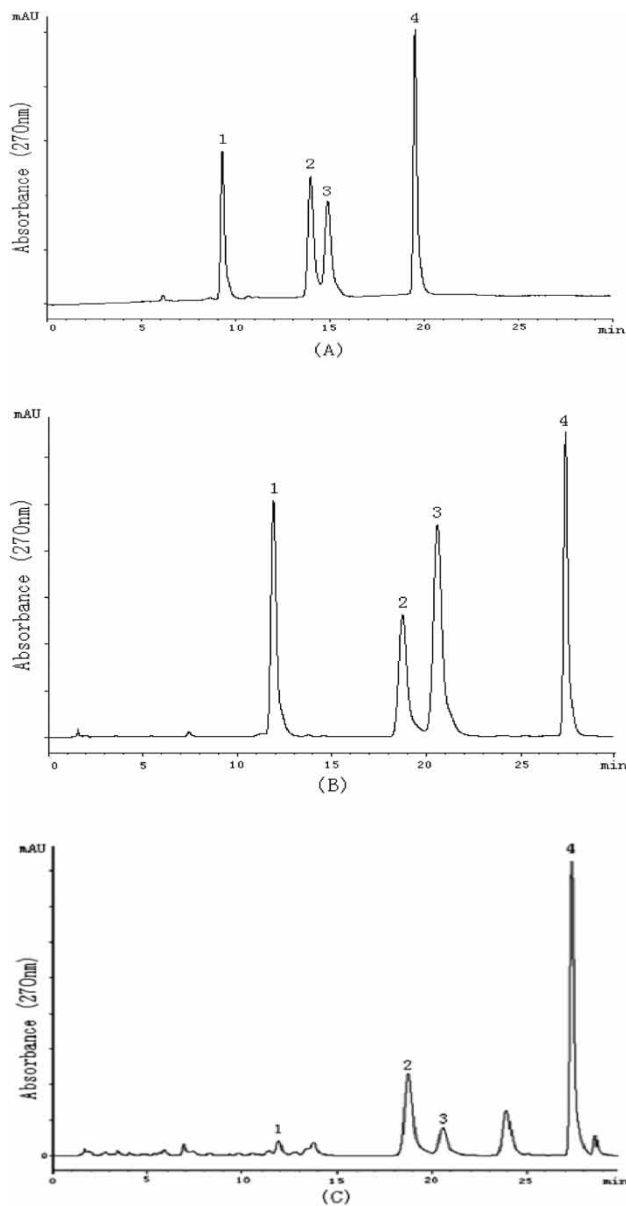

Figure 3. HPLC chromatograms of standard mixture and the SFE products of *Salvia miltiorrhiza*: (1) dihydrotanshinone I, (2) cryptotanshinone, (3) tanshinone I, and (4) tanshinone II_A. (A) The gradient elution of mobile phase was 65% (acetonitrile) in 0–17 min, and then linearly increased to 80% at the flow rate of 1.0 mL/min. (B) The gradient elution of mobile phase was 60% (acetonitrile) in 0–17 min, 60–80% (acetonitrile) in 17–25 min, 80% (acetonitrile) in 25–30 min. (C) New analytical method applied in the SFE products.

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could be baseline resolved (Figure 3(A)). Finally, we determined the mobile phase composition as water/acetonitrile (40:60). The gradient elution of mobile phase was 60% (acetonitrile) in 0–17 min, 60–80% (acetonitrile) in 17–25 min, 80% (acetonitrile) in 25–30 min. Under such chromatographic conditions, we can obtain good resolution of adjacent peaks within a short time (Figure 3(B)).

Method Validation

Linearity, Limits of Detection and Quantitation

The results are presentded in Table 1. The regression equation was calculated in the form of:

$$y = \mathrm{a}x + \mathrm{b}$$

where y and x were values of peak area and concentration of the standard compounds, respectively. The r^2 in Table 1 is referred to the correlation coefficient of the equation. All the standard compounds showed good linearity ($r^2 > 0.9994$) in the investigated concentration range for all the tanshinones.

LODs ranged from 2.10 to 6.64 ng for four investigated compounds, while LOQs ranged from 7.80 to 20.00 ng. The data of LOD and LOQ for each compound are also presented in Table 1.

Precision, Repeatability and Stability

In Table 2, the results related to intra-assay and inter-assay variability obtained from the assay of the standard mixture, are reported. The intra-assay precision expressed as RSDs ranged from 0.27% (Dihydrotanshinone I) to 1.26% (Tanshinone I). The inter-assay precision was determined by analyzing, in triplicate, a standard mixture at the same concentrations on three consecutive days with a RSD value ranging from 0.10% (Dihydrotanshinone I) to 0.29% (Tanshinone II_A).

To evaluate the repeatability, five different working solutions prepared from the same product of SFE were analyzed. The RSD values of the four investigated compounds were lower than 3.43%, suggesting that it has good repeatability.

Table 1. Linear regression data, LODs and LOQs of the investigated tanshinones from *Salvia miltiorrhiza*

Components	Linear range (μg/mL)	Regression equation[a]	Correlation factor (r^2)	LOD (ng)	LOQ (ng)
Dihydrotanshinone I	0.80–12.80	$y = 65.27x - 11.83$	0.9994	4.25	14.14
Cryptotanshinone	1.06–16.96	$y = 98.09x - 22.08$	0.9996	4.52	15.04
Tanshinone I	1.24–19.84	$y = 75.66x - 26.74$	0.9998	6.64	20.00
Tanshinone II_A	1.08–17.28	$y = 106.8x - 18.49$	0.9997	2.10	7.80

Note: [a]y: peak area; x: concentration (μg/mL).

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Table 2. Precision, repeatability and stability of the investigated compounds extracted from *Salvia miltiorrhiza*

	Precision							
	Intra-assay (n = 6)		Inter-assay (n = 3)		Repeatability (n = 5)		Stability (n = 9)	
Components	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)
Dihydrotanshinone I	7.38	0.27	7.26	0.10	4.59	1.38	4.57	0.51
Cryptotanshinone	10.25	0.15	10.14	0.19	9.87	2.22	9.95	0.39
Tanshinone I	12.16	1.26	12.14	0.21	11.37	3.43	11.6	0.85
Tanshinone II_A	11.22	0.25	11.14	0.29	11.85	1.77	11.9	0.40

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The data in Table 2 also shows that no significant degradation of the four investigated compounds was observed at room temperature for 72 h. The RSD values of the compounds ranged from 0.39% to 0.85%.

Recovery Test

As shown in Table 3, the recovery of the four investigated compounds ranged from 97.2% to 103.8%, and their RSD values were all less than 3.7%. Considering the results of the recovery test, the method is accurate and reliable.

Table 3. Recovery test of the developed method (n = 3)

Components	Quantity added (μg)	Quantity determined (μg)	Recovery (%)	RSD (%)
Dihydrotanshinone I	0.089	0.092	103.3	1.64
	0.145	0.144	99.3	2.09
	0.183	0.190	103.8	3.70
Cryptotanshinone	0.122	0.119	97.5	1.39
	0.203	0.203	100.0	0.66
	0.250	0.243	97.2	0.85
Tanshinone I	0.143	0.139	97.2	2.31
	0.238	0.244	102.5	0.65
	0.303	0.311	102.6	3.12
Tanshinone II_A	0.134	0.139	103.7	1.08
	0.222	0.224	100.9	0.43
	0.276	0.276	100.0	1.79

Table 4. Contents of four tanshinone compounds in SFE products under different extraction conditions

	Contents of four tanshinone compounds in the SFE products (%)			
No.	Dihydrotan-shinone I	Cryptotanshinone	Tanshinone I	Tanshinone II_A
1	2.225 ± 0.022	9.030 ± 0.018	3.348 ± 0.011	12.292 ± 0.233
2	2.350 ± 0.007	11.747 ± 0.210	2.978 ± 0.052	11.827 ± 0.079
3	1.385 ± 0.015	5.672 ± 0.090	2.157 ± 0.005	8.058 ± 0.110
4	0.949 ± 0.007	3.914 ± 0.058	1.528 ± 0.013	5.499 ± 0.077
5	1.519 ± 0.030	5.744 ± 0.105	3.206 ± 0.022	10.004 ± 0.167
6	3.118 ± 0.056	12.895 ± 0.153	3.798 ± 0.048	14.810 ± 0.234
7	2.772 ± 0.026	14.525 ± 0.189	7.553 ± 0.122	23.819 ± 0.375
8	2.306 ± 0.045	7.535 ± 0.098	6.905 ± 0.097	19.338 ± 0.276
9	1.067 ± 0.003	3.509 ± 0.032	2.930 ± 0.015	6.567 ± 0.036

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Application to Analysis of SFE Products

The developed analytical method was successfully applied to the simultaneous determination of dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone II_A in 9 samples of SFE products of *Salvia miltiorrhiza*, which were obtained under different conditions of extraction. Each sample was determined in triplicate. Peaks in the chromatograms were identified by comparing the retention times and on-line UV spectra with those of the standards. Retention times for the four compounds were 11.915, 18.764, 20.575, and 27.376 min, respectively (Figure 3(C)).

From the results presented in Table 4, it was found that the contents of four compounds varied greatly among the different samples. In all cases, tanshinone II_A was the main component, whose contents varied from 5.50 to 23.82% in 9 samples, with almost 4.33-fold variation. Similar variation could also be found from the other components. The reasons for the variation of contents can be the difference of extraction conditions, such as pressure, temperature, and extraction time. Because variations of the marker compounds may influence the quality and potency of the SFE products, it is necessary to develop an effective qualitative and quantitative method to evaluate the quality of the SFE products of *Salvia miltiorrhiza*. The assay of only one constituent cannot give a complete assessment of the SFE products. Thus, in this article, four compounds in SFE products were analyzed simultaneously to evaluate its quality.

CONCLUSION

A simple and efficient reverse-phase HPLC method was found to be more accurate, precise, and linear across the analytical range compared to the

former analytical methods reported. This method was specific for the simultaneous analysis of the four lipid soluble tanshinones from the product of SFE with good sensitivity, precision, and repeatability. This method can be applied as an improved quality control analysis for Danshen products and also facilitate the application of the SFE products of *Salvia miltiorrhiza* in the pharmaceutical industry.

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REFERENCES

1. Kasimu, R.; Tanaka, K.; Tezuka, Y.; Gong, Z.N.; Li, J.X.; Basnet, P.; Namba, T.; Kadota, S. Comparative study of seventeen Salvia plants: aldose reductase inhibitory activity of water and MeOH extracts and liquid chromatography-mass spectrometry (LC-MS) analysis of water extracts. Chem. Pharmaceut. Bull. **1998**, *46* (3), 500–504.
2. Lu, Y.; Yeap Foo, L. Polyphenolics of Salvia–a review. Phytochemistry **2002**, *59* (2), 117–140.
3. Wang, J.; Zhang, Y.; Yan, R. Fat reducing tea. CN Patent Number 1565210-A, 2002.
4. Wang, Z. Red sage leaf tea and its preparing process. CN Patent Number 1663413-A, 2005.
5. Xie, G. Method for preparing compound tea of ganoderma lucidum mycelium. CN Patent Number 1698850-A, 2005.
6. National Commission of Chinese Pharmacopoeia. *Pharmacopoeia of Peoples Republic of China*; Chemical Industry Press: Beijing, China, 2005; 52pp.
7. Lei, X.L.; Chiou, G.C. Cardiovascular pharmacology of Panax notoginseng (Burk) F.H. Chen and *Salvia miltiorrhiza*. Amer. J. Chin. Med. **1986**, *14* (3/4), 145–152.
8. Tian, G.; Zhang, T.; Zhang, Y.; Ito, Y. Separation of tanshinones from *Salvia miltiorrhiza* bunge by multidimensional counter-current chromatography. J. Chromatogr. A **2002**, *945* (1/2), 281–285.
9. Chang, H.M.; Cheng, K.P.; Choang, T.F.; Chow, H.F.; Chui, K.Y.; Hon, P.M.; Tan, F.W.L.; Yang, Y.; Zhong, Z.P.; Lee, C.M.; Chan, C.F.; Cui, Y.X.; Wong, H.C. Structure elucidation and total synthesis of new tanshinones isolated from *Salvia miltiorrhiza* bunge (Danshen). J. Org. Chem. **1990**, *55* (11), 3537–3543.
10. Lang, Q.Y.; Wai, C.M. Supercritical fluid extraction in herbal and natural product studies-a practical review. Talanta **2001**, *53* (4), 771–782.
11. Dean, J.R.; Liu, B.; Price, R. Extraction of tanshinone II_A from *Salvia miltiorrhiza* bunge using supercritical fluid extraction and a new extraction technique, phytosol solvent extraction. J. Chromatogr. A **1998**, *799* (1/2), 343–348.
12. Chen, B.; Zhu, M.; Xing, W.X.; Liu, L.L.; Wu, Y.T. SFE-CGC determination of tanshinone in *Salvia miltiorrhiza* bunge. Acta Pharmaceut. Sin. **2001**, *36* (1), 55–57.

13. Li, Y.C.; Zeng, J.Q.; Liu, L.M.; Jin, X.S. Extraction of three tanshinones from the root of *Salvia miltiorrhiza* bunge by supercritical carbon dioxide fluid and their analysis with high performance liquid chromatography. Chin. J. Chromatogr./Zhongguo hua xue hui **2002**, *20* (1), 40–42.
14. Zhou, J.M.; Liang, F.L.; Wu, G.X.; Yan, H. Studies on the extraction of ingredients from Chinese traditional medicine with CO_2 supercritical fluid. China J. Chin. Materia Medica **2003**, *28* (5), 413–417.
15. Cheng, C.H.; Hu, Y.C.; Yang, C.P.; Hou, F.; Mo, Q.W.; Jin, B.; Liu, H.C. A preparing method for Fufang Danshen tablet. CN Patent Number 1895353-A, 2007.
16. Hwang, J.I.; Kim, G.S.; Lee, S.H. Cosmetic composition containing *Salvia miltiorrhiza* bunge extract which is obtained by supercritical extraction and stabilized with nanoliposome. KR Patent Number 2004100080-A, 2004.
17. Xiao, X.L.; Gan, H.T.; Liao, Y.M.; Feng, X.T. Extracting progress for active compounds in *Salvia miltiorrhiza* by supercritical fluid extraction. CN Patent Number 1256142-A, 2000.
18. Lee, D.S.; Lee, S.H. Biological activity of dihydrotanshinone I: effect on apoptosis. J. Biosci. Bioengin. **2000**, *89* (3), 292–293.
19. Gu, M.; Zhang, G.; Su, Z.; Ouyang, F. Identification of major active constituents in the fingerprint of *Salvia miltiorrhiza* bunge developed by high-speed counter-current chromatography. J. Chromatogr. A **2004**, *1041* (1/2), 239–243.
20. Liu, A.H.; Lin, Y.H.; Yang, M.; Guo, H.; Guan, S.H.; Sun, J.H.; Guo, D.A. Development of the fingerprints for the quality of the roots of *Salvia miltiorrhiza* and its related preparations by HPLC-DAD and LC-MS^n. J. Chromatogr. B **2007**, *846* (1/2), 32–41.
21. Pan, X.; Niu, G.; Liu, H. Microwave-assisted extraction of tanshinones from *Salvia miltiorrhiza* bunge with analysis by high-performance liquid chromatography. J. Chromatogr. A **2001**, *922* (1/2), 371–375.
22. Tian, G.; Zhang, Y.; Zhang, T.; Yang, F.; Ito, Y. Separation of tanshinones from *Salvia miltiorrhiza* bunge by high-speed counter-current chromatography using stepwise elution. J. Chromatogr. A **2000**, *904* (1), 107–111.
23. Zhao, Y.; Chen, B.; Yao, S. Simultaneous determination of abietane-type diterpenes, flavonolignans and phenolic compounds in compound preparations of *Silybum marianum* and *Salvia miltiorrhiza* by HPLC-DAD-ESI MS. J. Pharmaceut. Biomed. Anal. 2005, 38 (3), 564–570.
24. Shi, Z.; He, J.; Yao, T.; Chang, W.; Zhao, M. Simultaneous determination of cryptotanshinone, tanshinone I, and tanshinone II_A in traditional Chinese medicinal preparations containing radix *Salvia miltiorrhiza* by HPLC. J. Pharmaceut. Biomed. Anal. **2005**, *37* (3), 481–486.
25. Zhang, J.; Wang, L.; Yuan, C.L.; Wang, X.Q.; Yu, Z.L. Simultaneous determination of four kinds of tanshinone in *Salviae miltiorrhizae* radix by reversed phase high-performance liquid chromatography. Chin. J. Analyt. Chem. **2005**, *33* (3), 355–358.

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