Simultaneous Determination of Danshensu, Protocatechuic Acid, Protocatechuic Aldehyde, Rosmarinic Acid and Salvianolic Acid B in Three Processing Products of White Flower *Salvia miltiorrhiza* by HPLC

Yuqin Li, Guirong You[*], Rui Duan, Ke Liu and Decai Wang

School of Pharmacy, Taishan Medical University, Taian 271016, P.R. China

*Corresponding author: Tel./Fax: +86 538 6229751; E-mail: gryou@tsmc.edu.cn

A reversed-phase high performance liquid chromatographic method was established for the simultaneous determination of five water-soluble components in three processing products of white flower Radix *Salvia miltiorrhiza*. The analysis was carried on a Yilite C_{18} column (250 mm × 4.6 mm, 5 μm). The mobile phase was a mixture of methanol and 0.5 % glacial acetic acid, employing gradient elution at a flow rate of 1 mL min^{-1}. Detection wavelength was at 286 nm. Regression equations revealed good linear relationship between the peak areas of the compounds and their concentrations (correlation coefficients: 0.9990-0.9999). The recoveries were between 98.91 and 104.4 %. The results indicated that protocatechuic aicd wasn't detected in the three processed products and the rest four contents presented the fresh < the wine fried < the carbonizing and provided the theory foundation for pharmacy and clinic.

Key Words: Danshensu, Protocatechuic acid, Protocatechuic aldehyde, Rosmarinic acid, Salvianolic acid B, *Salvia miltiorrhiza*.

INTRODUCTION

White flower *S. miltiorrhiza* (WFSM) is a variety of *S. miltiorrhiza* Bge. White flower *S. miltiorrhiza* has been grown only in mountain range and surrounding areas of Taishan of China [1]. The crude drugs and the processing products of roots of WFSM are widely used to for medical purpose in clinic[2]. The crude drugs have been used to treat palpitation-zhengzhong and insomnia due to febrile disease, boils, carbuncles, pain and swelling. The processing products have been used to treat stagnation and blood stasis, angina, chest tightness, palpitation and shortness of breath[3]. The effective components of WFSM could be classified as lipid-soluble and water-soluble. Recent years, research of water-soluble constituents in WFSM has been only focused on one or two compounds, which could not reflect the overall quality of WFSM[4,5]. Danshensu (DSS), protocatechuic acid (PAC), protocatechuic aldehyde (PAL), rosmarinic acid (RA) and salvianolic acid B (SAB) have been isolated and identified as the main constitutes in antiatherosclerosis, antiphlegmonosis, antioxidation and protecting myocardial damage in WFSM[6]. SAB can effectively restrain lipid peroxidation of the brain, liver and kidney[7-11]. And decoction with water is the conventional method of taking Chinese traditional medicine. Therefore, simultaneous determination of the five water-soluble constituents could control the overall quantity of WFSM.

In this report, a simple, rapid and accurate method was proposed for simultaneous determination DSS, PAC, PAL, RA and SAB in the three processing products of WFSM with HPLC.

EXPERIMENTAL

All separations were performed on a Shimadue LC-10ATvpPlus system (Shimadue Technologies, Suzhou, China) equipped with a SPD-10A VP detector. The system was controlled by an analytical workstation. The column was a Dalian Yilite C_{18} reserved-phase column (5 μm, 250 mm × 4.6 mm). The sample injection volume was 20 μL.

Danshensu, PAC, PAL, RA and SAB were purchased from the National Institute for Control of Biological and Pharmaceutical Products of China. HPLC grade methanol was purchased from Biaoshiqi Science and techonlogy Co., Ltd. (Tianjin, China). The roots of WFSM were collected from the planting base of Taishan Medical University and identified by prof. Gao Hongli. The processing products of WFSM (Fig. 1A-C) were made by lecturer Duanrui according to the Pharmacopoeia of People's Republic of China (2010 Edition)[12].

Preparation of calibration standard solutions: The stock solutions of DSS, PAC, PAL, RA and SAB were prepared with water, respectively and diluted to the appropriate concentration range (Table-1) for the establishment of calibration
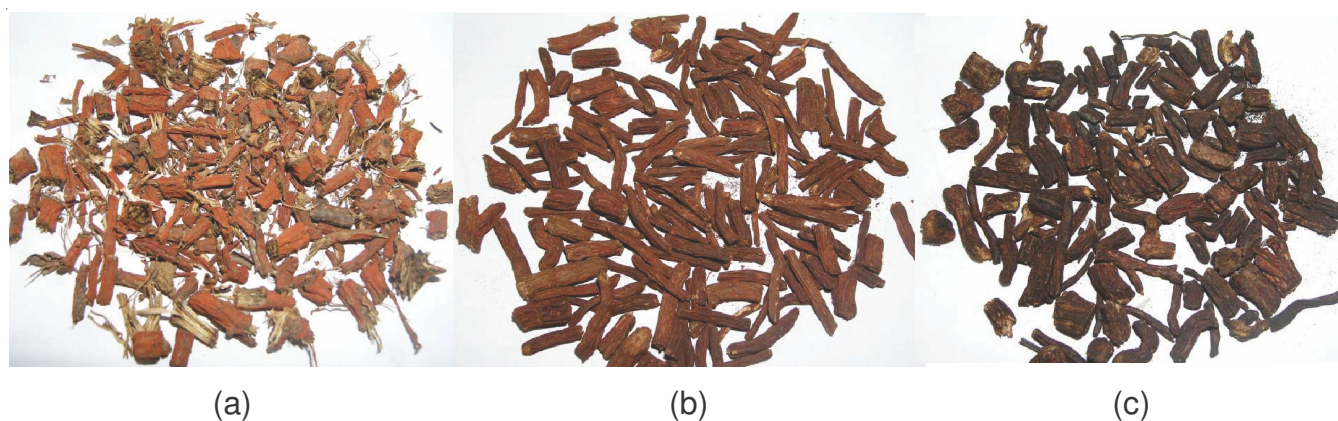
Fig. 1. Pictures of the three processing products of WFSM. (a) The crude of WFSM. (b) The wine-processing of WFSM. (c) The stir-frying of WFSM

TABLE-1
RESULTS OF REGRESSION ANALYSIS ON CALIBRATION CURVES AND THE DETECTION LIMITS

Analytes	Regression equation Y = a + bx	Linear range (μg mL^{-1})	Correlation coefficient	Detection limit[a] (μg mL^{-1})
DSS	Y = 15230X – 152.82	7.31-234	0.9995	0.028
PAC	Y = 44550X + 198.30	6.75-216	0.9999	0.026
PAL	Y = 93413X + 548.45	1.65-52.8	0.9990	0.0063
RA	Y = 37141X – 224.48	4.67-149.5	0.9998	0.018
SAB	Y = 19435X + 398.93	42.25-1352	0.9999	0.16

[a]Detection limit was defined as the concentration where the signal-to-noise ratio is 3.

curves. Each calibration curve was analyzed three times with six different concentrations.

Sample preparation: The powdered (100 mesh, 2.00 g) of the crude drugs of WFSM was placed in the 100 mL flask with plug. Then 20 mL water was added and lided. This mixture was oscillated for 1 h at 60 ºC and then, decocted for 0.5 h at 100 ºC. The extraction was cooled at room temperature and centrifugalized for 5 min at 4000 rpm. The upper solution was collected. The residue was repeatedly extracted with the same method. The upper solutions of the twice extrations were merged and dissolved with water to 50 mL. The method of extraction of the wine-processing and the stir-frying of WFSM were the same as above.

RESULTS AND DISCUSSION

Optimization of the separation conditions: To obtain good separation, various mobile phases, detection wavelengths and flow rates were investigated. When a Yilite C_{18} column was selected and detection wavelength was 286 nm, the best resolution was obtained with a gradient elution of 0.5 % (v/v) glacial acetic acid aqueous solution (A) and methonal (B). The elution programme was well optimized and conducted as follows: the first was 30 % B in the range of 0-6 min, the second linear gradient was 30-35 % B in the range of 10-30 min and the last one was 35-40 % B in the range of 30-40 min. Then the system was restored to initial conditions after 5 min. The solvent flow rate was 1.0 mL min^{-1}. The typical chromatogram for a standard mixture under the optimum conditions was shown in Fig. 2A.

Linearity, repeatability and detection limit: The linear relationships between the concentration X of the five analytes and the corresponding peak areas (Y) were listed in Table-1. The calibration curves exhibit excellent linear behaviour over the concentration range. The detection limits were also given in Table-1.

The repeatability of the peak area of DSS, PAC, PAL, RA and SAB were determined at three concentration levels (n = 5) in the ranges given above, respectively. The relative standard deviations (RSD) of the peak area of each peak were 0.47-1.57, 0.79-1.70, 0.26-1.59, 0.22-1.69 and 0.50-2.40 % (intra-day) and 0.72-1.99, 0.83-2.47, 0.68-1.85, 0.51-1.93 and 0.96-2.84 % (inter-day), respectively.
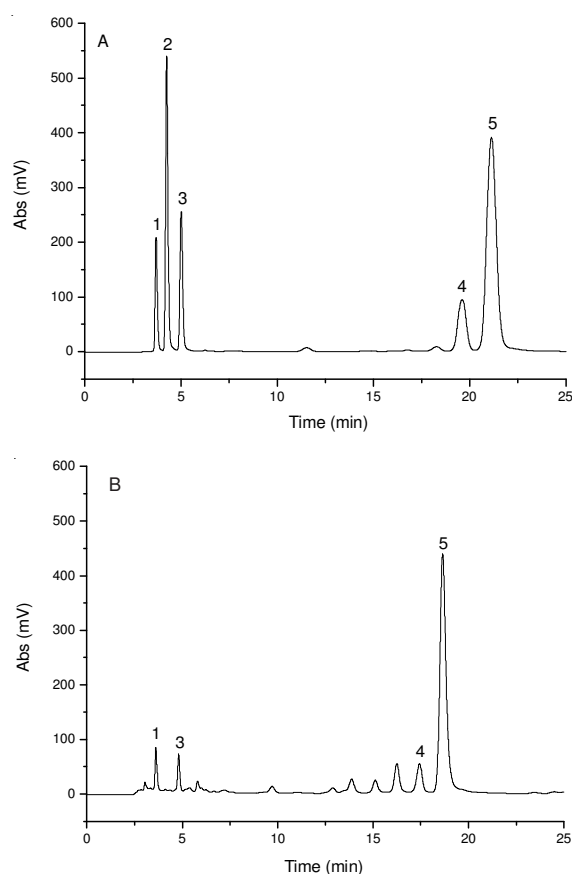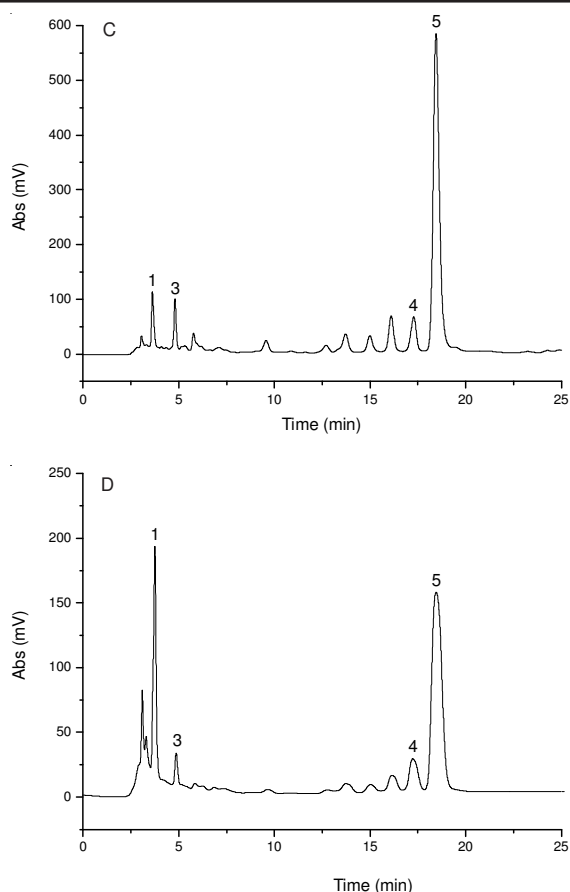
Fig. 2. HPLC chromatograms of the standards mixture solution and the samples. (A) The standard solution of 5 components. (B) The extraction of the WFSM crude. (C) The extraction of the WFSM wine-processing. (D) The extraction of the WFSM stir-frying. 1. Danshensu; 2. Protocatechuic acid; 3. Protocatechuic aldehyde; 4. Rosmarinic acid; 5. Salvianolic acid B

Application and recovery: The optimum conditions were applied to the separation and determination of DSS, PAC, PAL, RA and SAB in the three samples. The extraction of the crude was directly measured. The extraction of the wine processing was diluted three times and measured. The extraction of the stir-frying processing was diluted ten times and measured. The typical chromatograms were shown in Fig. 2B-D. The peaks were identified by comparison the migration times and by spiking the standards to the sample solution. The contents of the analytes found in the different kinds of herbs were given in Table-2.

TABLE-2
CONTENTS OF THE ANALYTES IN
THE DIFFERENT SAMPLES (n = 3)

Analytes	Crude	Wine prosessing	Stir-frying processing
DSS (%)	0.55	0.85	4.55
PAC (%)	ND[a]	ND	ND
PAL (%)	0.07	0.11	0.18
RA (%)	0.36	0.34	0.63
SAB (%)	6.02	6.94	7.95

[a]Not detection.

From Table-2, it was found that PAC was not detected, both in the crude WFSM and the two processing products. DSS, PAL, RA and SAB were all present in the three processing products of WFSM and the contents of DSS, PAL, RA and SAB presented an increasing trend from the crude to the wine fried and the stir-frying.

Recovery experiments were carried out by spiking the three samples (1.000 g powder) with different amounts (low, medium and high) of standards with known contents of marker compounds and the samples were treated according to the sample preparation procedure. The results were 98.91-104.4 % for DSS, 98.72-102.8 % for PAC, 99.82-103.5 % for PAL, 96.07-103.1 % for RA and 96.73-105.1 % for SAB, respectively.

Conclusion

In the present study, a HPLC method was established for the simultaneous determination of the five water-soluble components in three processing products of WFSM. The method is simple, accurate and effective and can be used to control the quality of the processing products. The results indicated that protocatechuic aicd wasn't detected in the three processed products and the rest four contents presented the fresh < the wine fried < the carbonizing stir-frying and provided the theory foundation for pharmacy and clinic.

REFERENCES

1. C.Q. Cao, L.R. Sun, H.X. Lou and M. Ji, *J. Chin. Med. Mater.*, **31**, 938 (2008).
2. X.M. Gao, Traditional Chinese Pharmacology, Chinese Publishing House of Traditional Chinese Medicine and Pharmacology, Beijing, p. 162 (2009).
3. D.J. Ye, S.C. Zhang and H. Wu, Science of Chinese Drug Processing, People's Medical Publishing House, Beijing, p. 136 (2011).
4. Y.J. Cui, Y.Q. Li, N.N. Yang, D.W. Li, X.M. Zhao and Z.L. Xia, *China Pharmacy*, **19**, 2592 (2008).
5. S.M. Mao, Y.L. Yan, F.X. Wang and F.J. Li, *Shandong Sci.*, **22**, 27 (2009).
6. Y.Q. Li, Y.J. Cui, X.M. Zhao, B.X. Jia and Y.X. Qi, *China J. Chin. Mater. Med.*, **36**, 1466 (2011).
7. C. Renzulli, F. Galvano, L. Pierdomenico, E. Speroni and M.C. Guerra, *J. Appl. Toxicol.*, **24**, 289 (2004).
8. A.M.J.J. Verweij-van Vught, B.J. Appelmelk, A.B.J. Groeneveld, M. Sparrius, L.G. Thijs and D.M. MacLaren, *Agents Actions*, **22**, 288 (1987).
9. K. Van, P.M. Kok, E.S. Kalter and J. Verhoef, Oxygen Free Radicals Shock, International Workshop, pp. 79-82 (1986).
10. K.P. Van Kessel, E.S. Kalter and J. Verhoef, *Agents Actions*, **17**, 375 (1986).
11. T. Shigematsu, S. Tajima, T. Nishikawa, S. Murad, S.R. Pinnell and I. Nishioka, *Biochim. Biophys. Acta*, **1200**, 79 (1994).
12. Pharmacopoeia of Peoples Republic of China, 2010 ed. Part I, The Chemical Industry Publishing House, Beijing, p. 70 (2010).