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Simultaneous quantification of six major phenolic acids in the roots of *Salvia miltiorrhiza* and four related traditional Chinese medicinal preparations by HPLC–DAD method

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Abstract

A high-performance liquid chromatographic method was applied to the determination of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, salvianolic acid B and salvianolic acid A in the roots of *Salvia miltiorrhiza* and four related traditional Chinese medicinal preparations. The six phenolic acids were simultaneously analyzed with a Zorbax Extend C_{18} column by gradient elution using 0.026% (v/v) phosphoric acid and acetonitrile as the mobile phase. The flow rate was 1 ml min^{-1}, and detection wavelength was set at 288 nm. The recovery of the method was in the range of 95.1–104.8%, and all the compounds showed good linearity ($r > 0.9997$) in a relatively wide concentration range. This assay was successfully applied to the determination of six major phenolic acids in 32 samples. The results indicated that the developed HPLC assay could be readily utilized as a quality control method for *S. miltiorrhiza* and its related traditional Chinese medicinal preparations.

Keywords: RP-HPLC; *Salvia miltiorrhiza*; Phenolic acid; Traditional Chinese medicinal preparation

1. Introduction

The dried roots of *Salvia miltiorrhiza* (Danshen), a traditional Chinese medicine, are widely used to treat coronary heart disease, cerebrovascular disease, bone loss, hepatitis, hepatocirrhosis and chronic renal failure [1–6]. There are a number of traditional Chinese medicinal preparations (TCMPs)-containing Danshen such as Fufang Danshen tablet (FDT), Compound Danshen dripping pills (CDDP), Danshen injection (DSI), Xiangdan injection (XDI), etc., among which Danshen is the major component. The TCMPs-containing Danshen were mainly used to treat coronary heart disease, heart-stroke, cerebrovascular diseases and cardiovascular diseases [7–13]. To ensure their clinical efficacy, quality control of *S. miltiorrhiza* and its related TCMPs is of great importance.

According to the pharmacological investigations, the active constituents of Danshen are divided into two groups: phenolic acids which are water soluble and tanshinones which are lipophilic [14–16]. In the past 20 years, the research attention has been focused on the phenolic acids of Danshen due to their notable pharmacological activities [17–20]. Up to now, quantitative determination of water soluble constituents in Danshen has been only focused on one or two compounds, which could not reflect the overall quality of Danshen. Based on the pharmacological research, protocatechuic aldehyde, rosmarinic acid and lithospermic acid have such biological activities as anti-atherosclerosis, anti-phlegmonosis, anti-oxidation and protecting myocardial damage [21–26]. While danshensu, salvianolic acid B and salvianolic acid A not only have the actions described above, but also are the characteristic constituents of *S. miltior-*

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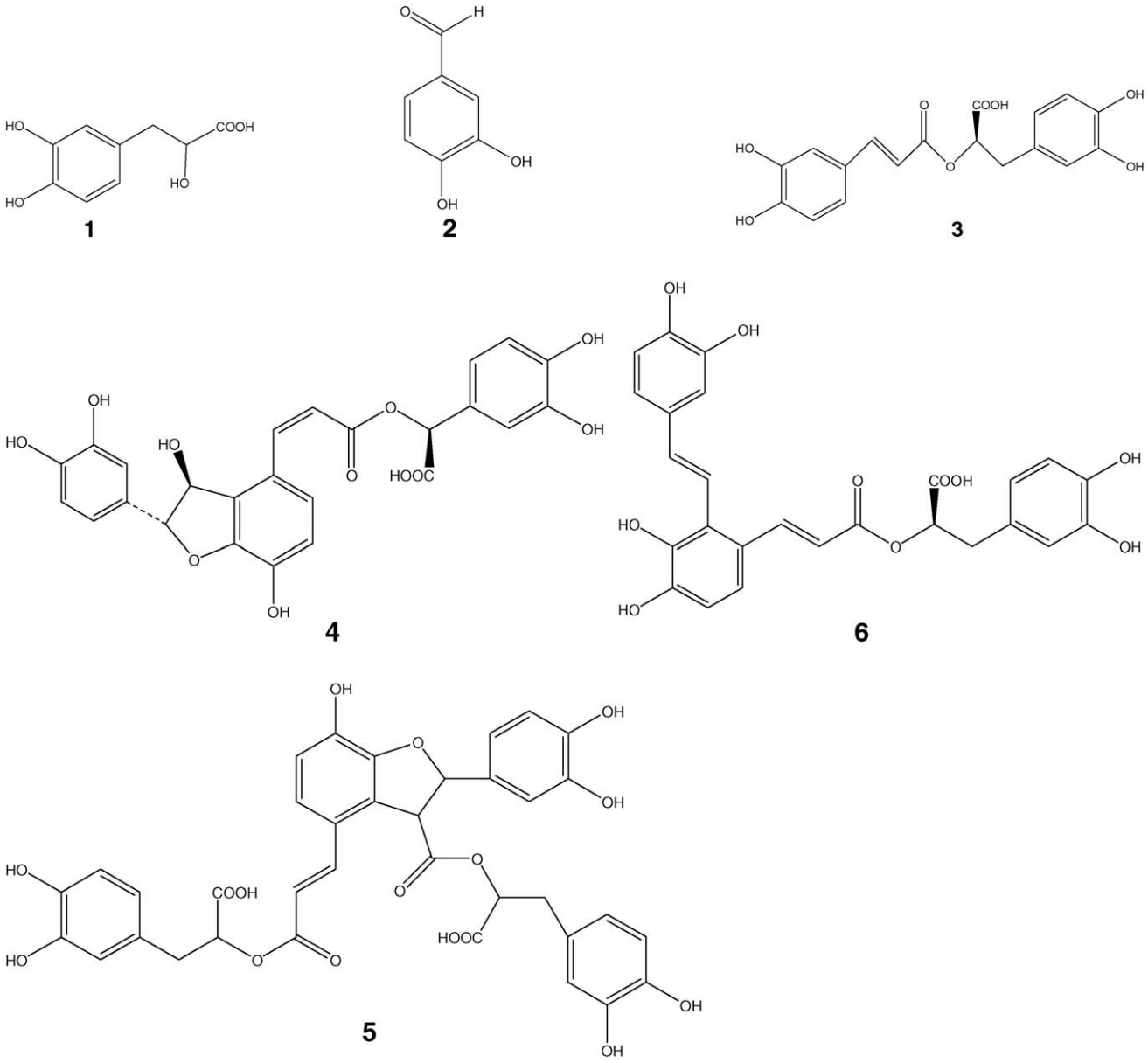

Fig. 1. The structures of standard compounds: **1**, danshensu; **2**, protocatechuic aldehyde; **3**, rosmarinic acid; **4**, lithospermic acid, **5**, salvianolic acid B; **6**, salvianolic acid A.

rhiza. Therefore, quantitative analysis of these constituents is significant for the quality control of *S. miltiorrhiza* and its related TCMPs.

Earlier we reported the metabolic studies of the total phenolic acids from the roots of *S. miltiorrhiza* and the major constituent—salvianolic acid B [27–29]. In the present paper, we intend to report the simultaneous quantification of the major phenolic acids (danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, salvianolic acid B and salvianolic acid A) (see Fig. 1) in *S. miltiorrhiza* and related TCMPs including FDT, CDDP, DSI and XDI (see Table 1) by reversed phase liquid chromatography. The results suggested that contents of the six major phenolic acids could be used to evaluate the quality of *S. miltiorrhiza* and its related TCMPs.

2. Experimental

2.1. Materials

All the samples were purchased from various drugstores around China (see Table 5). The roots of *S. miltiorrhiza* used to separate the standard compounds were obtained from Zhongjiang Danshen Cultivation Base in Sichuan province.

2.2. Chemicals and standards

HPLC grade acetonitrile and methanol (E. Merck, Darmstadt, Germany) were used for the HPLC analysis. Deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA). Phosphoric acid was of analytical grade from Beijing Beihua

Table 1
The TCMPs comprising of Danshen analyzed in the present study

Name of medicine	Dosage form	Composition	Function
Fufang Danshen tablet	Tablet	Radix Salviae Miltiorrhizae (450 g), Radix Notoginseng (141 g), Borneol (8 g)	Curing chest distress, angina pectoris, etc.
Compound Danshen dripping pills	Dripping pills	Radix Salviae Miltiorrhizae, Radix Notoginseng, Borneol	Curing chest distress, angina pectoral, etc.
Danshen injection	Injection	Radix Salviae Miltiorrhizae	Curing coronary artery disease, angina pectoris, chest distress, etc.
Xiangdan injection	Injection	Radix Salviae Miltiorrhizae (1000 g), Lignum Dalbergiae Odoriferae (1000 g)	Curing angina pectoris, myocardial infarction, etc.

Table 2
Calibration curves of six phenolic acids ($n = 4$)

Analyte	Retention time (min)	Standard curve	r^2	Test range (μg ml^{-1})	Limit of detection (μg ml^{-1})
Danshensu	10.30	$y = 5.2530x - 1.1146$	0.9998	10.84–346.9	0.15
Protocatechuic aldehyde	14.62	$y = 39.061x - 4.3277$	0.9998	0.690–24.15	0.04
Rosmarinic acid	27.50	$y = 19.156x - 8.4844$	0.9998	1.115–78.05	0.15
Lithospermic acid	28.38	$y = 11.272x - 7.9138$	0.9997	2.163–108.1	0.32
Salvianolic acid B	30.97	$y = 9.7908x - 30.393$	0.9999	13.50–810.0	0.43
Salvianolic acid A	33.73	$y = 33.616x + 15.493$	0.9997	2.530–88.55	0.18

y: peak area; x: concentration of analyte (μg ml^{-1}); limit of detection: S/N = 3.

Fine Chemicals Co. Ltd. (Beijing, China). Danshensu was purchased from the National Institute for Control of Biological and Pharmaceutical Products (China). Lithospermic acid was bought from Sigma (St. Louis, MO, USA). Protocatechuic aldehyde, rosmarinic acid, salvianolic acid B and salvianolic acid A were isolated from the roots of *S. miltiorrhiza* by the author. Their identities were confirmed by ^{1}H NMR, ^{13}C NMR and MS spectral analysis, and their purity was over 98% by HPLC analysis.

Table 3
Analytical results of intra-day and inter-day variability

Concentration (μg ml^{-1})	Intra-day ($n = 5$)			Inter-day ($n = 9$)		
	Found	R.S.D.[a] (%)	Accuracy (%)	Found	R.S.D. (%)	Accuracy[b] (%)
Danshensu						
21.68	21.51 ± 0.02	0.10	99.22	21.42 ± 0.10	0.46	98.80
216.80	214.81 ± 0.17	0.08	99.08	215.44 ± 1.53	0.71	99.37
325.20	320.48 ± 4.60	1.44	98.55	324.29 ± 3.06	0.94	99.72
Protocatechuic aldehyde						
0.79	0.77 ± 0.00	0.23	97.47	0.77 ± 0.01	0.65	97.09
10.35	10.18 ± 0.01	0.07	98.36	10.29 ± 0.13	1.27	99.40
19.32	19.02 ± 0.03	0.17	98.45	18.92 ± 0.15	0.79	97.93
Rosmarinic acid						
2.23	2.35 ± 0.00	0.00	105.38	2.34 ± 0.04	1.75	105.11
33.45	32.04 ± 0.04	0.12	95.8	33.62 ± 1.28	3.81	100.50
62.44	60.30 ± 0.06	0.10	96.57	63.08 ± 1.34	2.12	101.03
Lithospermic acid						
4.33	4.51 ± 0.01	0.22	104.16	4.49 ± 0.17	3.83	103.63
43.25	42.91 ± 0.08	0.19	99.21	42.61 ± 2.01	4.73	98.51
90.83	93.90 ± 0.05	0.06	103.38	94.38 ± 2.87	3.04	103.91
Salvianolic acid B						
27.00	26.95 ± 0.06	0.24	99.81	27.49 ± 0.60	2.17	101.80
405.00	391.17 ± 0.39	0.10	96.59	395.30 ± 2.88	0.73	97.60
756.00	722.97 ± 0.78	0.11	95.63	757.04 ± 23.36	3.04	100.14
Salvianolic acid A						
2.53	2.42 ± 0.01	0.58	95.66	2.55 ± 0.11	4.31	100.79
37.95	37.53 ± 0.08	0.22	98.89	36.79 ± 0.86	2.34	96.94
70.84	69.15 ± 0.23	0.34	97.61	67.73 ± 1.46	2.16	95.66

[a] R.S.D. (%) = (S.D./mean) × 100.
[b] Recovery (%) = [(mean of measured concentration − spiked concentration)/spiked concentration] × 100.

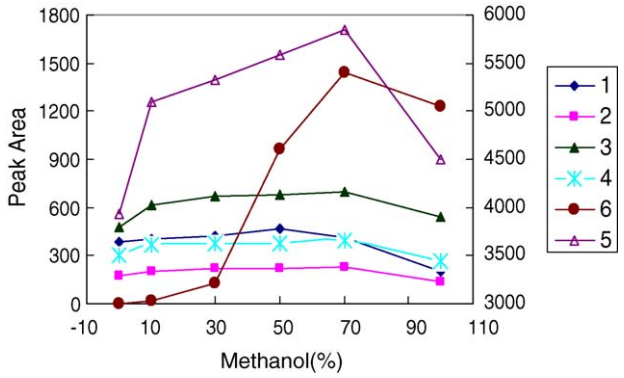

Fig. 2. Extraction efficiency of different solvents: **1**, danshensu; **2**, protocatechuic aldehyde; **3**, rosmarinic acid; **4**, lithospermic acid; **5**, salvianolic acid B; **6**, salvianolic acid A.

2.3. Apparatus and chromatographic conditions

An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) comprised a quaternary solvent delivery system, an on-line degasser, an auto-sampler, a column temperature controller and DAD detector coupled with an analytical workstation. The column configuration was an Agilent Zorbax Extend C_{18} reserved phase column (5 μm, 250 mm × 4.6 mm) coupled with an Agilent Zorbax Extend C_{18} guard column (5 μm, 10 mm × 4.6 mm). The sample injection volume was 10 μl.

Detection wavelength was set at 288 nm, the flow rate was 1.0 ml min^{-1} and the column temperature was maintained at 20 °C. The mobile phase was gradient elution which was mixed with solvent A (0.026% aqueous phosphoric acid, v/v) and B (acetonitrile). The gradient program was as follows: initial 0–20 min, linear change from A–B (98:2, v/v) to A–B (77:23, v/v); next 20–35 min, linear change to A–B (71.5:28.5, v/v).

An FW100 pulverizer (24,000 rpm, 460 W) from the Tianjin City Taisite Instrument Co. Ltd. (Tianjin, China) was used to comminute Danshen. The quantitative filter paper (No. 201, 9 cm) from Hangzhou Fuyang Special Industry Co. Ltd. (Hangzhou, China) and the organic membrane (13 mm, 0.45 μm) from Tianjin Tengda Filtration Instrument Co. Ltd. (Tianjin, China) were used.

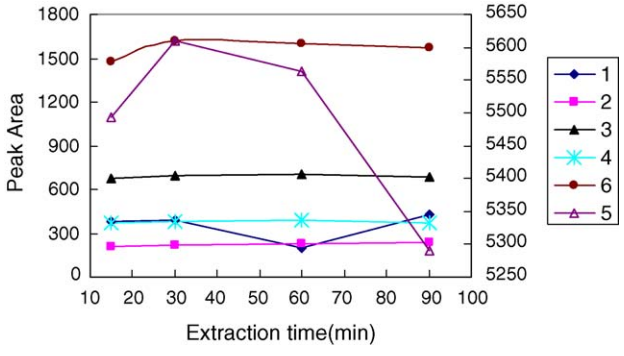

Fig. 3. Extraction efficiency of different extraction time: **1**, danshensu; **2**, protocatechuic aldehyde; **3**, rosmarinic acid; **4**, lithospermic acid; **5**, salvianolic acid B; **6**, salvianolic acid A.

Table 4
Analytical results of recoveries ($n = 4$)

Phenolic acid spiked (μg/ml)	Found (μg/ml)	R.S.D.[a] (%)	Recovery[b] (%)
Danshensu			
21.68	20.61 ± 0.53	2.57	95.05
130.08	129.84 ± 1.53	1.18	99.81
220.37	217.90 ± 1.77	0.81	98.88
Protocatechuic aldehyde			
0.69	0.68 ± 0.01	0.84	98.99
6.90	6.75 ± 0.06	1.48	97.83
13.80	13.12 ± 0.33	2.52	95.07
Rosmarinic acid			
2.23	2.31 ± 0.07	2.88	103.74
22.30	23.30 ± 0.23	0.98	104.48
55.75	54.42 ± 0.69	1.26	97.61
Lithospermic acid			
4.33	4.39 ± 0.09	2.09	101.44
43.25	42.85 ± 1.30	3.04	99.08
69.20	71.9 ± 0.84	1.17	103.90
Salvianolic acid B			
27.00	28.30 ± 0.24	0.84	104.78
216	219.99 ± 1.77	0.80	101.85
351	364.80 ± 4.34	1.19	103.93
Salvianolic acid A			
5.06	5.07 ± 0.05	0.93	100.26
25.30	24.74 ± 0.78	3.13	97.80
50.60	51.79 ± 0.58	1.11	102.36

[a] R.S.D. (%) = (S.D./mean) × 100.
[b] Recovery (%) = [(mean of measured concentration − spiked concentration)/spiked concentration] × 100.

2.4. Preparation of standard solutions

A 50% of aqueous methanol stock solution-containing danshensu (**1**), protocatechuic aldehyde (**2**), rosmarinic acid (**3**), lithospermic acid (**4**), salvianolic acid B (**5**) and salvianolic acid A (**6**) was prepared and diluted to appropriate concentration range for the establishment of calibration curves. Each calibration curve was analyzed four times with six different concentrations using the same HPLC condition as described in Section 2.3.

2.5. Preparation of sample solutions

The dried roots of *S. miltiorrhiza* was comminuted (100 mesh). After the coating was scraped off, FDT was also comminuted. CDDP was ground into pieces. Each solid sample (0.300 g) was suspended in 70% methanol (10 ml) and extracted in a regurgitant bath for 1 h. Then the extraction solutions were prepared by the method of weight relief. As to Danshen injection and Xiangdan injection, 1 ml of liquid sample was diluted directly to 6 ml with deionized water. For determination of phenolic acids, the solution was filtered through a membrane (0.45 μm) and then injected into HPLC.

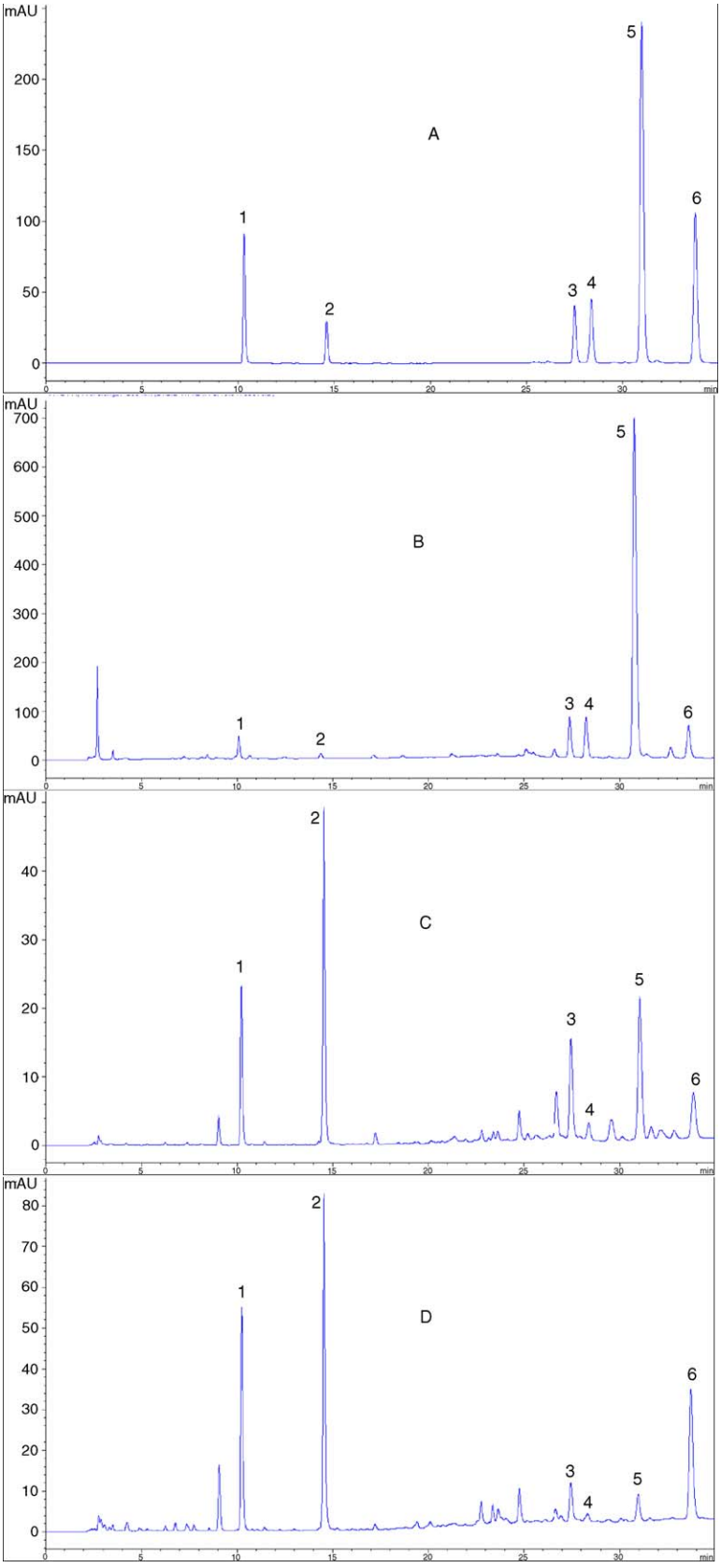

Fig. 4. Representative HPLC chromatograms of (A) standard solution at high concentration, (B) Fufang Danshen tablet (Hebei, China, 9919), (C) Danshen injection (Guangzhou, China, 040811), (D) Xiangdan injection (Shanghai, China, 200408214), (E) Compound Danshen dripping pills (Tianjing, China, 2004218) and (F) Danshen (Henan, China, 2003). **1**, Danshensu; **2**, protocatechuic aldehyde; **3**, rosmarinic acid; **4**, lithospermic acid; **5**, salvianolic acid B; **6**, salvianolic acid A.

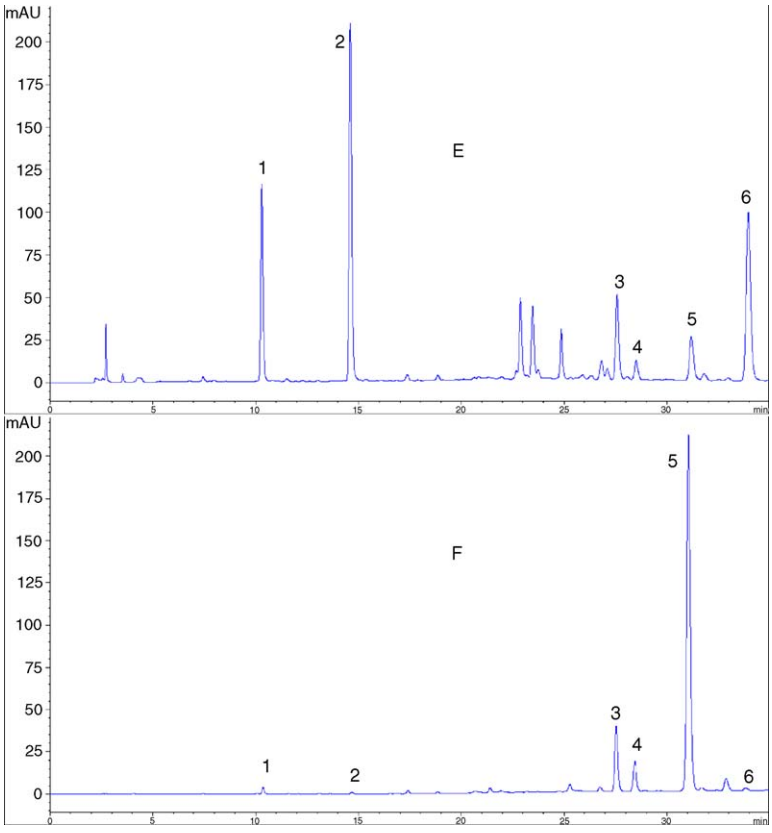

Fig. 4. (*Continued*).

3. Results and discussion

3.1. Chromatographic conditions

A good separation condition should satisfy the need that the analyzed peaks have baseline separation with adjacent peaks within a short analysis time as far as possible. To obtain the chromatograms with the good separation, fixed phase, mobile phase, column temperature, detection wavelength and flow rate were, respectively, investigated.

For phenolic acids, the fixed phase of Zorbax Extend C_{18} column was better than BDS-Hypersil C_{18}, YMC-Pack ODS-A C_{18} and Luna C_{18} columns. Various mixtures of water and acetonitrile were used as mobile phase but separation was not satisfactory. According to literatures [30–32], acid could achieve better separation for phenolic acids since it reduces the ionization of phenol, phenolic hydroxyl and carboxyl groups. It was also suggested that separation was better when column temperature was kept at 20 °C than 15, 30 and 40 °C. The most suitable flow rate was found to be at 1.0 ml min^{-1}. According to the UV absorption maxima of the phenolic acids, the chromatograms were recorded at 288 nm.

3.2. Sample extraction conditions

In order to obtain quantitative extraction of solid sample, variables involved in the procedure such as solvent, extraction method and extraction time were optimized.

Methanol, 70% methanol, 50% methanol, 30% methanol, 10% methanol and water were employed as extraction solvents. Pure water could not extract the salvianolic acid B and salvianolic acid A completely while danshensu and protocatechuic aldehyde could not be efficiently extracted by pure methanol. From the extraction efficiency of the different solvents (see Fig. 2), it is clear that, when 70% methanol was employed, the peak areas of the six phenolic acids reached the highest values. Therefore, 70% methanol was selected as the extraction solvent. After comparing several extraction methods such as ultrasonic, soaking and regurgitation, regurgitation was found to be the most suitable extraction method.

Then the optimal extraction time was investigated. Solid sample (0.300 g) was extracted with 10 ml 70% MeOH for 15, 30, 60 and 90 min, respectively. The peak areas of the marker constituents obtained by different extraction times were shown in Fig. 3, from which one can see that all the phenolic acids were almost completely extracted within 30 min. Hence 30 min was selected as the optimal extraction time.

From above experiment, the most suitable extraction method of phenolic acids was regurgitant extraction by 10 ml of 70% MeOH for 30 min.

3.3. Calibration curves and the limit of detection

The external standard method was used to get the regression equations. The calculated results were given in Table 2. In

Table 5
The contents of six phenolic acids in *Radix Salvia miltiorrhizae* (Danshen) and four related TCMPs ($n = 3$)

No.	Batch number	Province of collected place	Content (%)[f] ($n = 3$)					
			Danshensu	Protocatechuic aldehyde	Rosmarinic acid	Lithospermic acid	Salvianolic acid B	Salvianolic acid A
1[a]	30902	Guangdong	1.03 ± 0.00	0.08 ± 0.00	1.56 ± 0.00	1.05 ± 0.02	17.70 ± 0.16	0.22 ± 0.00
2[a]	31207	Beijing	10.00 ± 0.08	5.24 ± 0.01	1.18 ± 0.02	0.95 ± 0.02	19.66 ± 0.06	1.16 ± 0.01
3[a]	40210	Henan	5.21 ± 0.08	0.60 ± 0.00	1.64 ± 0.01	0.87 ± 0.01	19.68 ± 0.09	1.99 ± 0.06
4[a]	30207	Zhejiang	2.01 ± 0.04	0.12 ± 0.00	0.83 ± 0.00	1.81 ± 0.03	21.71 ± 0.30	1.03 ± 0.03
5[a]	20040561	Yunnan	2.67 ± 0.03	0.36 ± 0.00	1.17 ± 0.01	1.29 ± 0.03	24.16 ± 0.15	1.10 ± 0.02
6[a]	2003110	Heirongjiang	4.24 ± 0.04	0.36 ± 0.00	1.11 ± 0.01	1.69 ± 0.03	23.22 ± 0.23	1.67 ± 0.04
7[a]	40401	Jiangxi	1.93 ± 0.00	0.15 ± 0.00	0.82 ± 0.02	0.81 ± 0.03	12.49 ± 0.18	1.01 ± 0.03
8[a]	20301	Jiangxi	0.73 ± 0.03	0.09 ± 0.00	0.75 ± 0.01	1.27 ± 0.02	21.14 ± 0.43	0.11 ± 0.00
9[a]	40107	Sichuang	1.00 ± 0.02	0.02 ± 0.00	1.07 ± 0.01	1.46 ± 0.02	23.82 ± 0.48	0.17 ± 0.00
10[a]	40427	Beijing	8.30 ± 0.01	0.44 ± 0.00	1.30 ± 0.02	0.90 ± 0.02	15.36 ± 0.29	2.19 ± 0.03
11[a]	40301	Guangdong	2.96 ± 0.03	0.10 ± 0.00	1.50 ± 0.02	0.89 ± 0.02	11.51 ± 0.17	0.84 ± 0.02
12[a]	30901	Guangdong	0.77 ± 0.01	0.11 ± 0.00	1.97 ± 0.01	0.84 ± 0.02	13.62 ± 0.20	0.13 ± 0.00
13[a]	31211	Guangdong	1.30 ± 0.03	0.06 ± 0.00	0.71 ± 0.02	1.44 ± 0.02	18.67 ± 0.30	0.53 ± 0.00
14[a]	40309	Guangxi	0.45 ± 0.01	nd[g]	0.71 ± 0.02	1.17 ± 0.00	19.33 ± 0.18	nd[g]
15[a]	40403	Jiangxi	1.84 ± 0.01	0.19 ± 0.00	0.95 ± 0.02	0.86 ± 0.00	14.84 ± 0.18	0.77 ± 0.01
16[a]	40501	Guangdong	0.48 ± 0.01	0.05 ± 0.00	0.46 ± 0.00	0.52 ± 0.02	11.03 ± 0.15	0.03 ± 0.00
17[a,*]	4051005	Guangdong	2.57 ± 0.02	0.19 ± 0.00	1.13 ± 0.18	1.02 ± 0.03	16.24 ± 0.23	1.26 ± 0.03
18[a,*]	4011008	Guangdong	2.70 ± 0.02	0.18 ± 0.00	0.92 ± 0.02	1.07 ± 0.01	15.89 ± 0.26	1.57 ± 0.03
19[a,*]	4041012	Guangdong	2.70 ± 0.02	0.17 ± 0.00	0.97 ± 0.00	0.93 ± 0.00	14.99 ± 0.28	1.16 ± 0.01
20[a,*]	4021008	Guangdong	2.76 ± 0.02	0.17 ± 0.00	0.92 ± 0.02	0.88 ± 0.00	13.80 ± 0.20	1.23 ± 0.03
21[a,*]	4031018	Guangdong	2.74 ± 0.02	0.19 ± 0.00	0.96 ± 0.01	1.02 ± 0.02	16.06 ± 0.16	1.27 ± 0.03
22[a,*]	3111005	Guangdong	2.73 ± 0.02	0.17 ± 0.00	1.04 ± 0.01	1.11 ± 0.01	16.00 ± 0.12	1.48 ± 0.02
23[a,*]	4081001	Guangdong	2.97 ± 0.02	0.17 ± 0.00	1.01 ± 0.01	1.07 ± 0.01	15.82 ± 0.24	1.26 ± 0.02
24[a,*]	3121020	Guangdong	2.87 ± 0.01	0.18 ± 0.00	1.00 ± 0.01	1.04 ± 0.03	15.23 ± 0.26	1.31 ± 0.01
25[a]	20040407	Liaoning	4.38 ± 0.06	0.54 ± 0.00	1.26 ± 0.03	1.70 ± 0.03	26.71 ± 0.36	2.05 ± 0.04
26[a]	403006	Jiangshu	6.81 ± 0.03	0.33 ± 0.00	1.58 ± 0.02	1.40 ± 0.00	22.81 ± 0.31	3.34 ± 0.05
27[a]	403006	Xingjiang	0.97 ± 0.03	0.06 ± 0.00	0.61 ± 0.01	1.24 ± 0.01	16.35 ± 0.13	0.31 ± 0.00
28[a]	20030903	Shanxi	3.38 ± 0.02	0.24 ± 0.00	1.38 ± 0.01	1.04 ± 0.03	16.87 ± 0.22	2.42 ± 0.06
29[b]	40915	Guangdong	18.91 ± 0.10	6.41 ± 0.07	5.13 ± 0.02	1.94 ± 0.04	19.76 ± 0.37	1.55 ± 0.01
30[c]	40710	Shanghai	22.61 ± 0.13	5.47 ± 0.07	1.70 ± 0.07	0.85 ± 0.01	3.41 ± 0.05	3.65 ± 0.03
31[d]	20030618	Tianjing	6.89 ± 0.04	2.02 ± 0.03	1.16 ± 0.01	0.69 ± 0.00	1.67 ± 0.02	1.79 ± 0.05
32[e]	040913	Henan	0.59 ± 0.01	0.03 ± 0.00	2.37 ± 0.01	2.00 ± 0.03	30.30 ± 0.13	0.25 ± 0.00

[a] Fufang Danshen tablet.
[b] Danshen injection.
[c] Xiangdan injection.
[d] Compound Danshen dripping pill.
[e] drug of Danshen.
[f] Content = mean ± S.D. ($n = 3$), unit of solid sample is mg g^{-1} drug, unit of liquid sample is mg 10 ml^{-1} drug.
[g] nd: not detected.
[*] The samples are produced from the same factory.

the regression equation $y = ax + b$, x is referred to the concentration of the standard compounds (μg ml^{-1}), y to the peak area, a is the intercept of the straight line with y-axis and b is the slope of the line. The r in Table 2 is referred to the correlation coefficient of the equation. All the standard compounds showed good linearity ($r > 0.9997$) in a relatively wide concentration range.

The limit of detection (LOD) was measured based on the method described by the International Conference on Harmonization [1]. The standard solution was diluted with solvent to provide appropriate concentrations. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. LOD was in the range of 0.04–0.43 μg ml^{-1} (see Table 2).

3.4. System suitability test

The precision test was carried out by the intra-day and inter-day variability. Three different concentration solutions (low, medium and high) of authentic standards were prepared. The quantity of each analyte was obtained from corresponding calibration curve. The relative standard deviation (R.S.D.) was taken as a measure of precision. The intra-day variability was examined within 1 day in five times and the result showed that R.S.D. of intra-day variability was in the range of 0.00–1.44% (see Table 3). The inter-day precision was calculated from nine determinations over 3 days for each concentration and the results were in the range of 0.46–4.73% (see Table 3).

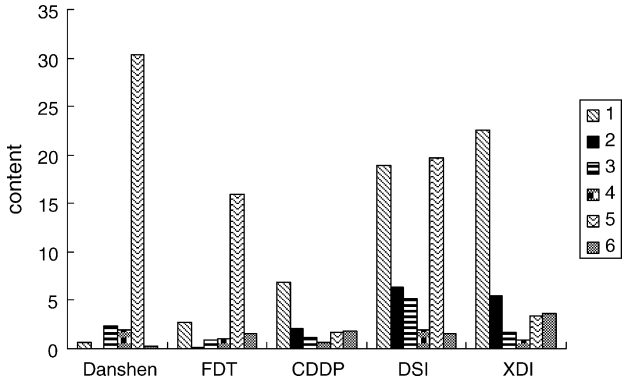

Fig. 5. The ratio of six phenolic acids in Danshen and related TCMPs. **1**, Danshensu; **2**, protocatechuic aldehyde; **3**, rosmarinic acid; **4**, lithospermic acid; **5**, salvianolic acid B; **6**, salvianolic acid A.

The recovery test was carried as followings: three different quantities (low, medium and high) of authentic standards were added into samples. The resultant samples were extracted and analyzed as described in Section 3.5. The quantity of each analyte was subsequently obtained from the corresponding calibration curve. The recovery of the six standards ranged from 95.1 to 104.8% (see Table 4). From the results of precision test and recovery test, it was known that the method manifested good precision and accuracy.

For stability test, the same sample solution was analyzed every 12 h in 3 days at the room temperature, and the analytes were found to be rather stable within 72 h (R.S.D. < 5.4%).

3.5. Sample analysis

The five categories of samples were prepared as described in Section 2.5. A volume of 10 μl of the filtered solution of each sample was injected into the instrument. 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 time for compounds **1**, **2**, **3**, **4**, **5** and **6** were 10.30, 14.62, 27.50, 28.38, 30.97 and 33.73 min, respectively (see Fig. 4). The content of each analyte was calculated from the corresponding calibration curve.

The established method has been applied to the determination of the six phenolic acids in Danshen and four Danshen-contaning TCMPs. The contents of phenolic acids in Danshen, FDT, CDDP, XDI and DSI were reported for the first time. The contents of the six phenolic acids in the 28 FDT samples including 21 samples from different manufacturers and 8 samples from the same manufacturer were shown in Table 5, from which one can see that the contents of the six phenolic acids in FDT varied greatly among the different manufacturers. The variation of contents may be derived from the different quality of the raw material, the difference of production procedure, storage, transportation, etc. From the data in Table 5, it could also be inferred that the contents in different batches of FDT produced by the same manufacturer were relatively consistent.

The contents of the six phenolic acids in Danshen and related TCMPs were also shown in Table 5 and Fig. 5, from which it is obvious that the ratio of the contents of six phenolic acids in Danshen and four related TCMPs varied drastically. The reason for such ratio variation might be due to the different processing procedure of Danshen during the manufacturing process.

4. Conclusion

This was the first report on the simultaneous determination of six major phenolic acids in the samples of roots of *S. miltiorrhiza* and four Danshen-containing TCMPs. A simple, rapid and accurate assay method was successfully established. The results suggested that this HPLC method could be considered as good quality criteria to control the quality of *S. miltiorrhiza* and its related TCMPs.

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