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Improved quality control method for Danshen products—Consideration of both hydrophilic and lipophilic active components

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Abstract

The current study intends to provide an improved quality control analysis for Danshen product—a representative herbal product with known active components that are both hydrophilic and lipophilic in nature. A simple HPLC method with photodiode-array (PDA) ultraviolet detection was developed for the simultaneous determination of three major lipophilic components (cryptotanshinone, tanshinone I and tanshinone IIA) and three major hydrophilic components (danshensu, protocatechuic aldehyde and salvianolic acid B) of Danshen (*Salvia miltiorrhiza*). These six components were successfully separated using Radial-pak C18 cartridge with the elution gradient consisting of 0.5% acetic acid in water and 0.5% acetic acid in acetonitrile at a flow rate of 1 ml/min. The intra-day and inter-day precisions of the analysis were within 2.32 and 2.0%, respectively. The detection limits were 0.02, 0.01, 0.01, 0.05, 0.005 and 0.02 μg/ml for cryptotanshinone, tanshinone I, tanshinone IIA, danshensu, protocatechuic aldehyde and salvianolic acid B, respectively. The developed method has been applied to the simultaneous determination of above six major components in Fufang Danshen Tablet and Dripping Pill products by extraction with methanol and water. It has been demonstrated that salvianolic acid B and danshensu are the major components among the eight commercial Fufang Danshen products studied. The current developed method with methanol as extraction solvent provides a simple and efficient method for simultaneous detection of both lipophilic and hydrophilic major components in Danshen products.

Keywords: Cryptotanshinone; Tanshinone I; Tanshinone IIA; Danshensu; Protocatechuic aldehyde; Salvianolic acid B; HPLC

1. Introduction

Danshen, the dried root of *Salvia miltiorrhiza* (Fam. Labiatae), is used as a traditional Chinese medicine for promoting circulation and improving blood stasis. It is also widely used for the treatment and prevention of coronary heart diseases, hyperlipidemia [1–3] and cerebrovascular diseases [4]. At present, numerous Danshen products are commercially available, especially in China. These consist of tablets, capsules, granules, injection preparations, oral liquids, dripping pills and sprays of either Danshen or Fufang Danshen which is the composite of *Salvia miltiorrhiza*, *Panax notoginseng* and *Cinnamomum camphora* [5–7]. Among all the available dosage forms of Danshen, Fufang Danshen Tablet and Fufang Danshen Dripping Pill, the two most commonly used ones in China, have already been officially listed in the Chinese Pharmacopoeia.

The chemical constituents of Danshen include both lipophilic and hydrophilic components. As shown in Fig. 1, the major lipophilic components are cryptotanshinone, tanshinone I and tanshinone IIA [8–10] and the major hydrophilic components include danshensu (salvianic acid A), protocatechuic aldehyde and salvianolic acid B [11]. Various in vitro and in vivo pharmacological activity studies have demonstrated that both lipophilic and hydrophilic components can improve microcirculation [12], dilate the coronary arteries [13], increase the blood flow and prevent myocardial ischemia [14–16].

For the quality control of medicinal products, chemical markers are often utilized. In Chinese Pharmacopoeia 2000, danshensu has been selected as the marker for the quality control of Fufang Danshen Dripping Pill (content of danshensu per pill should not be less than 0.08 mg), and tanshinone IIA is the marker for Fufang Danshen Tablet (content of tanshinone IIA per tablet should not be less than 0.2 mg) [17]. However, according to the Chinese Pharmacopoeia 2005, tanshinone IIA and salvianolic acid B have both been selected as the marker components for the quality control of Fufang Danshen Tablet [18].

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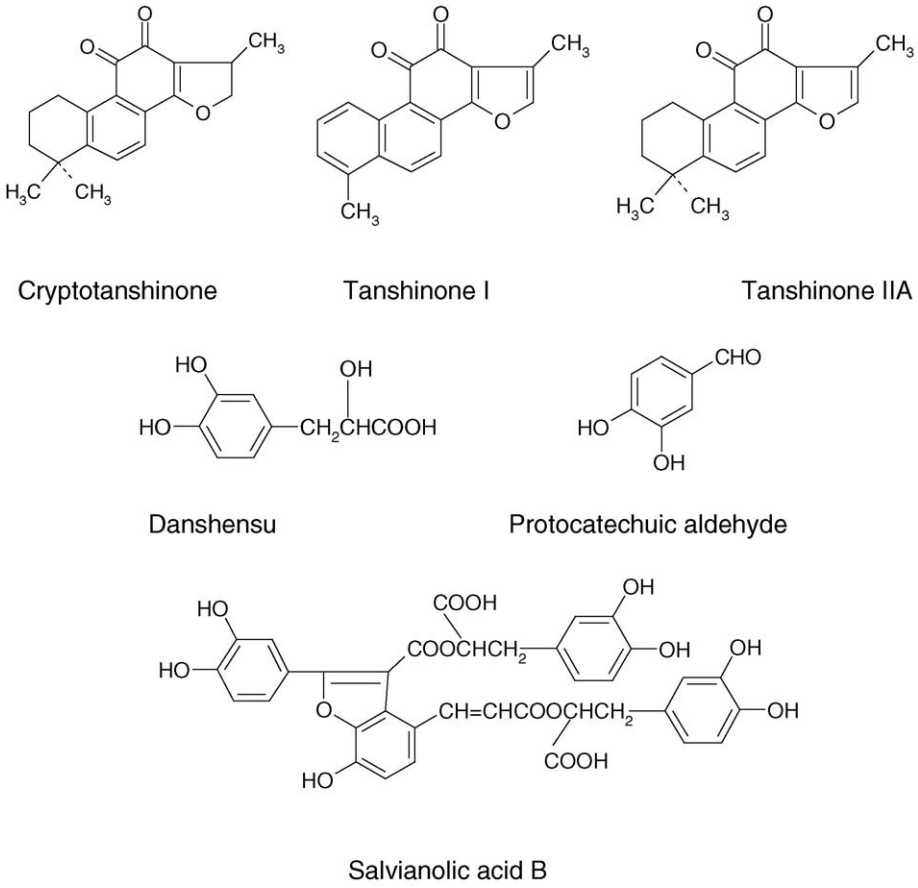

Fig. 1. Chemical structures of the six studied Danshen components.

This latest recommendation reflects the current consideration of the need to include both lipophilic and hydrophilic markers for the quality control of Danshen products.

As a result of the long history use of Danshen, a number of assays have been developed for detecting chemical markers in various Danshen products. One of the recent HPLC study was able to simultaneously determine three lipophilic components (namely cryptotanshinone, tanshinone I and tanshinone IIA) in Danshen medicinal preparations after methanol extraction [19]. Hu et al. developed a multi-component fingerprinting method using reversed phase HPLC and LC-MS-MS for detection of rosmarinic acid, salvianolic acid B and tanshinone IIA in Danshen crude drugs and processed medicinal materials using methanol extraction followed by boiled water extraction [20]. Moreover, assays have been developed to detect lipophilic components and hydrophilic components in Danshen products using separated HPLC assays with water extraction for hydrophilic components and methanol extraction for lipophilic components [21,22]. In both Chinese Pharmacopoeia 2000 and 2005, methanol was recommended as the extraction solvent for the quantifications of danshensu in Fufang Danshen Dripping Pill and tanshinone IIA in Fufang Danshen Tablet. In Chinese Pharmacopoeia 2005, water was recommended as the extraction solvent for the determination of salvianolic acid B.

In summary, previous studies focused on using separate assays or extraction solvents for hydrophilic and lipophilic markers present in Danshen products. For herbal products, such as Danshen products that contain both hydrophilic and lipophilic active markers, a rapid and simple assay method for simultaneous detection of both types of markers will be desirable. The present report presents a simple HPLC method for the simultaneous determination of essential hydrophilic and lipophilic components of Danshen including danshensu, protocatechuic aldehyde, salvianolic acid B, cryptotanshinone, tanshinone I and tanshinone IIA. This method will be applied to evaluate the contents of the studied six components in eight commercial available Danshen products including seven brands of Fufang Danshen Tablets and one brand of Danshen Dripping Pill. In addition, the effect of the extraction solvent will be investigated to find the simplest and most efficient sample preparation method for Danshen products.

2. Material and methods

2.1. Chemicals and instrument

Cryptotanshinone, tanshinone I, salvianolic acid B and protocatechuic aldehyde were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Tanshinone IIA, sodium danshensu was purchased from School of Pharmacy, Fudan University (Shanghai, PR China). Griseofulvin, used as internal standard, was purchased from

Sigma (St. Louis, MO, USA). Fufang Danshen Dripping Pill and seven brands of Fufang Danshen Tablets (named Tablet A–G) were purchased from pharmacy stores in mainland China and Hong Kong, PR China. Acetonitrile (HPLC grade) were obtained from Labscan Asia, Thailand. Methanol (HPLC grade) was obtained from TEDIA company, Inc., USA. Acetic acid (analytical grade) was obtained from BDH Laboratory supplies, England. All other reagents were of at least analytical grade and used without further purification. Distilled and deionized water was used for the preparation of all solutions.

The liquid chromatographic system used was a Waters HPLC system (Waters, Milford, MA, USA) equipped with 2695 solvent delivery module and a 996 photodiode-array (PDA) UV detector. The chromatographic separation of the six compounds and internal standard was achieved by using a reversed-phase HPLC column (Radial-pak C_{18} cartridge, 10 cm × 8 mm i.d.; 4 μm particle size, Waters) protected by a pre-column filter (Nove-pak C_{18} Guard-pak, Waters).

2.2. Chromatographic condition

The elution gradient for HPLC analysis consisted of two solvent compositions: 0.5% acetic acid in acetonitrile (solvent A) and 0.5% acetic acid in water (solvent B). Gradient elution was carried out according to the following program: solvent A was increased from 5 to 50% in the first 20 min, then increased to 65% in the next 2 min, held for 18 min and then returned to 5% in 5 min. The sample injection volume was 100 μl. The column and auto-sampler were set at ambient temperature. The flow rate was set at 1 ml/min. The eluent was monitored by a UV detector at the wavelength of 280 nm for all the studied compounds.

2.3. Preparation of standard solutions and calibration curves

A 100.0 μg/ml of cryptotanshinone and tanshinone IIA, 20.0 μg/ml of tanshinone I, 500.0 μg/ml of sodium danshensu, protocatechuic aldehyde and salvianolic acid B were prepared separately in methanol. Standard stock solution containing cryptotanshinone, tanshinone I, tanshinone IIA, sodium danshensu, protocatechuic aldehyde and salvianolic acid B was prepared by mixing and diluting their original stock solutions with 0.5% acetic acid in methanol/water (1:1) to reach the final concentrations of 12.0, 6.0, 6.0, 20.0, 2.0 and 10.0 μg/ml, respectively. Working standard solutions and the quality control sample solutions at high, medium and low concentrations were prepared by diluting the standard stock solutions with 0.5% acetic acid in methanol/water (1:1). These solutions were all kept in brown glass bottles and stored at −20 °C. The internal standard solution consisted 5 μg/ml of griseofulvin in methanol.

A 0.5 ml of working standard calibration solutions were mixed with 10 μl of internal standard solution. Calibration curves were constructed by plotting the peak-area ratio of each analyte/internal standard versus analyte concentration. The studied concentration ranges were 0.5–15.0 μg/ml for danshensu, 0.05–1.5 μg/ml for protocatechuic aldehyde, 0.25–7.5 μg/ml for salvianolic acid B, 0.3–9.0 μg/ml for cryptotanshinone and 0.15–4.5 μg/ml for both tanshinone I and tanshinone IIA.

2.4. Validation of method

Validation of the HPLC method was performed by determining the intra-day, inter-day accuracy and precision. The quality control samples were analyzed in a set of five on a single assay day to determine intra-day precision and accuracy, and analyzed in duplicate on each of three separate days to determine inter-day precision and accuracy. The quality control samples at low, medium and high concentrations (danshensu: 1.0, 4.0, 12.0 μg/ml; protocatechuic aldehyde: 0.1, 0.4, 1.2 μg/ml; salvianolic acid B: 0.5, 2.0, 6.0 μg/ml; cryptotanshinone: 0.6, 2.4, 7.2 μg/ml; tanshinone I: 0.3, 1.2, 3.6 μg/ml; tanshinone IIA: 0.3, 1.2, 3.6 μg/ml) were used. The limit of detection was defined as the lowest concentration of the analyte resulting in a signal-to-noise ratio of 3:1.

2.5. Sample preparations for Danshen products

In the current study, both methanol and water were used to extract all the six studied components from Fufang Danshen Dripping Pill and Tablets in order to compare the effect of extraction solvent on the product quantification so as to determine the most efficient extraction procedure applicable for detecting the above markers from Danshen products.

2.5.1. Extraction of Fufang Danshen Dripping Pill

Five accurately weighed pills were extracted with methanol or water in a 10 ml brown v-flask by sonication for 2 h. The final volume was made up to 10 ml after the solution being cooled down. The mixture was then filtered and the filtrate was diluted appropriately with 0.5% acetic acid in methanol/water (1:1) for HPLC analysis.

2.5.2. Extraction of Fufang Danshen Tablets

The sugar coats of 10 tablets were washed off with water and the tablets were dried followed by being grinded into fine powder. Based on preliminary tests, extractions with methanol and water were conducted at different solvent to powder ratios in order to compromise the detection of both hydrophilic (usually in high contents) and lipophilic (usually in low contents) components in Danshen products. For extraction with methanol, 1 g of the dried powder was accurately weighed into a 25 ml brown v-flask followed by addition of methanol and sonication for 15 min. The final volume was built up to 25 ml after the solution being cooled down. For extraction with water, 0.15 g of the dried powder was sonicated with water for 30 min in a 50 ml brown v-flask. The final volume of the solution was made up to 50 ml after being cooled down. All the prepared mixtures in the flasks were shaken well before being filtered. The filtrate was diluted appropriately with 0.5% acetic acid in methanol/water (1:1) before being injected into HPLC for analysis.

2.6. Recovery test

Extractions of either the Fufang Danshen Dripping Pill or grinded Fufang Danshen Tablet powder with and without prior addition of each standard compound ranging from low, medium and high levels were both conducted. The extraction recoveries of each compound were calculated as the percentage of the net amount of each compound obtained after extraction from that had been added prior to the extraction.

3. Results and discussions

Polarities of the three lipophilic Danshen components are extremely different from that of the three hydrophilic components. Therefore, a gradient elution with 0.5% acetic acid in acetonitrile and water was carried out to achieve a relative shorter retention time with a sufficient resolution for all analytes. Fig. 2 represents typical chromatograms of the six Danshen components in standard solution, extract of Fufang Danshen Dripping Pill and Fufang Danshen Tablet, respectively. Under the current experimental conditions, the retention times for danshensu, protocatechuic aldehyde, salvianolic acid B, griseofulvin (internal standard), cryptotanshinone, tanshinone I and tanshinone IIA were at 6.8, 9.7, 15.1, 23.1, 32.9, 34.2 and 41.6 min, respectively.

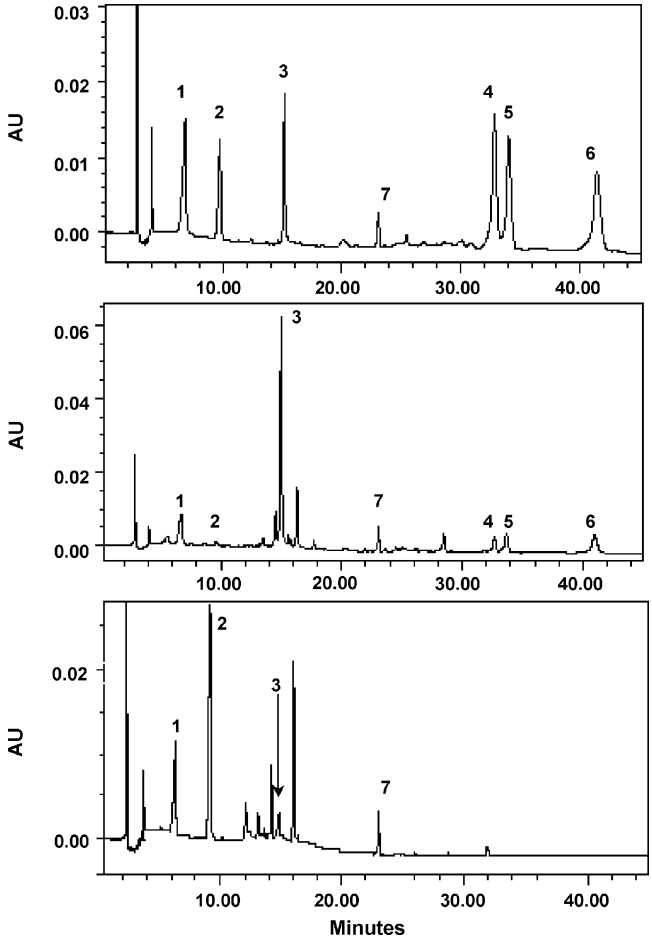

Fig. 2. HPLC/UV chromatogram of the six studied Danshen components in standard solution (upper), extract of Fufang Danshen Tablet (middle) and Fufang Danshen Dripping Pill (lower): (1) danshensu; (2) protocatechuic aldehyde; (3) salvianolic acid B; (4) cryptotanshinone; (5) tanshinone I; (6) tanshinone IIA; (7) internal standard.

Table 1
Linearity and sensitivity for detection of six Danshen components with HPLC/UV analysis

Analyte	Concentration range (μg/ml)	Correlation coefficient (r^2)	LOD (μg/ml)
Danshensu	0.50–15.00	0.9987	0.05
Protocatechuic aldehyde	0.05–1.50	0.9993	0.005
Salvianolic acid B	0.25–7.50	0.9994	0.02
Cryptotanshinone	0.30–9.00	0.9991	0.02
Tanshinone I	0.15–4.50	0.9996	0.01
Tanshinone IIA	0.15–4.50	0.9998	0.01

As shown in Table 1, calibration curves of the tested compounds were linear over the studied concentration ranges with $r^2 > 0.999$ for all six analytes. The detection limits for danshensu, protocatechuic aldehyde, salvianolic acid B, cryptotanshinone, tanshinone I and tanshinone IIA were determined to be 0.05, 0.005, 0.02, 0.02, 0.01 and 0.02 μg/ml, respectively, which were similar to that from previous studies when hydrophilic and lipophilic components from Danshen were measured separately [19,22].

Analytical accuracy and precision data are shown in Table 2 and are expressed as mean detected concentration and relative standard deviation (R.S.D. %). The precisions of the six analytes at low to high concentrations were within 2.32 and 2.0% for intra-day and inter-day assays, respectively.

Mean extraction recoveries of the six analytes from Fufang Danshen Dripping Pill (Table 3a) and Fufang Danshen Tablet (Table 3b) at low, medium and high concentration levels varied from 82.3 to 100.0% when methanol extraction was used for the sample preparations. When water was used as the extraction medium for Fufang Danshen Dripping Pill (Table 3a) and Fufang Danshen Tablet (Table 3b), three hydrophilic components (danshensu, protocatechuic aldehyde and salvianolic acid B) were detected with similar extraction recoveries as that from the methanol extraction, whereas the three non-polar components (cryptotanshinone, tanshinone I and tanshinone IIA) were not detectable. The above recovery test results suggested that the methanol extraction method was efficient enough for the determination of both lipophilic and hydrophilic Danshen components in commercial Danshen preparations.

The developed HPLC method has been applied to determine the six Danshen components in seven brands of Fufang Danshen Tablets and one brand of Fufang Danshen Dripping Pill. Table 4 shows the content of each component in the above eight preparations obtained with both methanol and water as extraction solvents.

For Fufang Danshen Tablets, our results with methanol extraction were similar to that from Zhang et al. [21]. Danshensu and salvianolic acid B are found to be the major components in the eight studied Danshen products. Danshensu was the component with the highest content in one of the Fufang Danshen Tablet (Tablet D), whereas salvianolic acid B was the one in highest

Table 2
Intra-day and inter-day accuracy and precision for the determination of six Danshen components with HPLC/UV analysis

Analyte	Nominal conc. (μg/ml)	Intra-day ($n=5$)			Inter-day ($n=3$)		
		Mean detected conc. (μg/ml)	Deviation (%)	R.S.D. (%)	Mean detected conc. (μg/ml)	Deviation (%)	R.S.D. (%)
Danshensu	12.00	12.04	0.29	1.16	12.13	1.12	0.79
	4.00	4.04	0.49	1.01	4.11	2.67	2.00
	1.00	1.02	1.71	2.32	1.02	2.30	1.46
Protocatechuic aldehyde	1.20	1.20	−0.02	0.38	1.20	0.35	0.96
	0.40	0.41	2.29	0.46	0.41	1.69	1.57
	0.10	0.10	1.33	0.48	0.10	2.46	1.17
Salvianolic acid B	6.00	5.98	−0.41	1.72	5.92	−1.33	1.49
	2.00	2.02	0.87	0.65	2.01	0.28	1.01
	0.50	0.51	1.71	0.05	0.50	0.48	0.93
Cryptotanshinone	7.20	7.22	0.32	1.19	7.17	−0.44	0.80
	2.40	2.39	−0.39	0.32	2.39	−0.62	1.08
	0.60	0.61	1.83	1.04	0.61	0.83	0.76
Tanshinone I	3.60	3.63	0.87	0.29	3.59	−0.35	0.94
	1.20	1.21	0.21	1.13	1.20	0.25	0.76
	0.30	0.30	−0.78	0.29	0.30	−0.40	0.42
Tanshinone IIA	3.60	3.62	0.66	1.14	3.59	−0.40	0.45
	1.20	1.20	0.46	0.96	1.20	−0.07	0.73
	0.30	0.30	1.30	1.18	0.30	0.29	0.70

amount in the other six brands of Fufang Danshen Tablets. Substantial amount of the lipophilic components, such as cryptotanshinone, tanshinone I and tanshinone IIA, were also detected in Fufang Danshen Tablets after methanol extraction, but with much lower amount than the hydrophilic components such as danshensu or salvianolic acid B. Among the three lipophilic components, tanshinone IIA was the major component found from all seven brands of Fufang Danshen Tablets. Table 4 also indicated that only three hydrophilic components are detectable with water extraction method. The contents of danshensu, protocatechuic aldehyde and salvianolic acid B obtained from water extraction were similar to that obtained from methanol extraction.

In addition to various brands of Fufang Danshen Tablet, the only commercial available brand of Fufang Danshen Dripping Pill was tested. Since only aqueous extract of Danshen was used

Table 3a
Extraction recoveries of the three hydrophilic Danshen components obtained from water and methanol extraction of Fufang Danshen Dripping Pill ($n=3$)

Compound	Extraction solvent	Concentration		Recovery (%)
		Spiked (μg/ml)	Detected (μg/ml)	
Danshensu	Methanol	80.0	75.4 ± 2.2	94.3 ± 2.7
		40.0	37.2 ± 1.27	93.1 ± 3.2
		2.0	1.9 ± 0.05	93.5 ± 2.3
	Water	80.0	79.2 ± 0.6	99.0 ± 0.8
		40.0	39.6 ± 0.2	99.1 ± 0.5
		2.0	2.0 ± 0.03	97.5 ± 1.3
Salvianolic acid B	Methanol	10.0	9.7 ± 0.1	97.4 ± 0.5
		5.0	4.9 ± 0.04	97.7 ± 0.7
		1.0	1.0 ± 0.01	95.0 ± 1.0
	Water	10.0	9.8 ± 0.1	98.1 ± 0.6
		5.0	4.9 ± 0.05	98.6 ± 0.9
		1.0	1.0 ± 0.01	97.7 ± 0.6
Protocatechuic aldehyde	Methanol	25.0	24.9 ± 0.04	99.7 ± 0.2
		10.0	9.9 ± 0.1	98.9 ± 0.6
		0.04	0.04 ± 0.001	97.4 ± 2.4
	Water	25.0	25.0 ± 0.04	99.8 ± 0.1
		10.0	9.9 ± 0.08	98.6 ± 0.8
		0.04	0.04 ± 0.001	97.4 ± 2.4

Table 3b
Extraction recoveries of the six Danshen components obtained from water and methanol extraction of Fufang Danshen Tablet ($n = 3$)

Compound	Extraction solvent	Concentration		Mean recovery (%)
		Spiked (μg/ml)	Detected (μg/ml)	
Danshensu	Methanol	450.0	409.5 ± 20.9	91.0 ± 4.6
		200.0	181.2 ± 17.6	90.6 ± 8.8
		23.2	19.1 ± 2.1	82.3 ± 8.9
	Water	33.2	33.4 ± 1.4	100.6 ± 4.1
		16.0	15.8 ± 1.1	98.8 ± 7.0
		1.8	1.7 ± 0.1	94.4 ± 5.6
Salvianolic acid B	Methanol	1300.0	1201.2 ± 51.7	92.4 ± 4.0
		600.0	568.8 ± 18.2	94.8 ± 3.0
		80.0	73.3 ± 5.7	91.6 ± 7.1
	Water	101.8	100.9 ± 8.7	99.1 ± 8.5
		50.0	49.7 ± 5.7	99.4 ± 7.4
		6.0	5.8 ± 0.7	96.8 ± 11.0
Protocatechuic aldehyde	Methanol	5.2	5.1 ± 0.3	98.4 ± 5.1
		2.6	2.6 ± 0.2	99.2 ± 8.0
		0.4	0.39 ± 0.07	97.5 ± 10.9
	Water	0.4	0.38 ± 0.03	95.0 ± 8.5
		0.2	0.2 ± 0.01	98.7 ± 2.9
		0.04	0.04 ± 0.0	96.2 ± 1.4
Cryptotanshinone	Methanol	77.2	75.7 ± 6.2	99.8 ± 8.0
		38.0	38.0 ± 2.1	100.0 ± 5.4
		11.2	11.1 ± 0.8	99.1 ± 7.2
	Water	5.8	ND	NA
		3.0	ND	NA
		0.8	ND	NA
Tanshinone I	Methanol	63.2	62.9 ± 4.1	99.5 ± 6.4
		32.0	31.7 ± 1.3	99.1 ± 3.9
		7.6	7.3 ± 0.5	96.1 ± 5.9
	Water	4.8	ND	NA
		2.4	ND	NA
		0.6	ND	NA
Tanshinone IIA	Methanol	82.4	82.4 ± 3.5	100.0 ± 4.2
		40.0	39.9 ± 3.4	99.7 ± 8.5
		25.6	25.4 ± 1.9	99.3 ± 7.3
	Water	6.2	ND	NA
		3.0	ND	NA
		2.0	ND	NA

ND, not detectable; NA, not applicable.

Table 4
Content of major Danshen components in commercial Fufang Danshen Dripping Pill and Tablet products using methanol or water as extraction solvent

Products	Component content (mg/pill or mg/tablet)											
	Danshensu		Protocatechuic aldehyde		Salvianolic acid B		Cryptotanshinone		Tanshinone I		Tanshinone IIA	
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water
Dipping Pill	0.17	0.16	0.05	0.05	0.02	0.02	ND	ND	ND	ND	ND	ND
Tablet A	2.00	2.12	0.04	0.04	4.14	4.13	0.44	ND	0.57	ND	0.74	ND
Tablet B	0.21	0.25	ND	0.03	0.72	0.78	0.10	ND	0.13	ND	0.23	ND
Tablet C	0.33	0.36	ND	ND	0.53	0.56	0.15	ND	0.08	ND	0.27	ND
Tablet D	2.87	2.99	ND	ND	1.06	1.12	0.11	ND	0.05	ND	0.22	ND
Tablet E	0.46	0.48	0.02	0.02	0.87	0.91	0.16	ND	0.09	ND	0.25	ND
Tablet F	0.76	0.79	0.04	0.04	10.17	10.49	0.58	ND	0.14	ND	0.37	ND
Tablet G	1.43	1.50	0.02	0.03	3.10	3.22	0.24	ND	0.20	ND	0.25	ND

ND, not detectable.

as the raw material for the Dripping Pill preparations [23], it was expected that only hydrophilic components being detectable regardless of the extraction solvents used. The contents of the three detectable hydrophilic components obtained from water extraction were also found to be similar to that from methanol extraction. Moreover, Danshensu was detected as component in highest content, which once again confirmed the selection of it as the chemical marker for the quality control in the pharmacopoeia.

Essential quality control markers are considered to be the minimum number of markers that not only represent essential components with sufficient quantity but also can be determined readily by an available assay method. Comparing our approaches used to test essential quality control markers in Danshen products versus that have been published in previous studies as well as Chinese Pharmacopoeia, it was found that the other studies only focused on using either lipophilic markers or hydrophilic markers with separate extraction methods for detecting various kinds of components in Danshen products. Consistent with the previous findings, our current study indicated that Danshensu and Salvianoic acid B are the two major components in the tested Danshen products. Moreover, similar to what have been found by Zhang et al. [21] and Shi et al. [19], our study also demonstrated that the amount of the six components did vary a lot among different brands of Danshen preparations.

In conclusion, this study describes the development, validation and application of a HPLC method for simultaneous determination of the six major lipophilic and hydrophilic components in commercial Danshen preparations. The developed method is not only simple and efficient with excellent accuracy, precision and reproducibility, but also provides a simultaneous determination of the six major Danshen components and can be used for the improved in-process quality control of Danshen products.

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References

[1] J.Y. Ma, Tian Jin Zhong Yi 15 (1998) 24.
[2] W.Y. Ma, J.S. Yang, C.C. Gong, L. Liang, Jie Fang Jun Yao Xue Xue Bao 17 (2001) 47–48.
[3] S.K. Cui, G.H. Li, Xian Dai Zhong Xi Yi Jie He Za Zhi 11 (2003) 1029–1030.
[4] J.M. Mao, Z.Q. Gan, Jie Fang Jun Yao Xue Xue Bao 17 (2001) 49–50.
[5] X.Q. Liu, X.T. Luo, X.A. Ouyang, Shi Zhen Guo Yi Guo Yao 12 (2001) 474–475.
[6] X.R. Hu, F.M. Cao, Zhong Guo Xin Yi Yao 2 (2003) 54–55.
[7] Y.R. Ge, L. Fang, J. Li, Zhong Cheng Yao 24 (2002) 872–875.
[8] D.Y. Kong, Zhong Guo Yi Yao Gong Ye Za Zhi 20 (1989) 279–285.
[9] G.H. Zheng, H. Kakisawa, Zhong Guo Yao Xue Za Zhi 24 (1989) 6–10.
[10] D.G. Cai, Zhong Guo Zhong Yao Za Zhi 16 (1991) 376–377.
[11] L.N. Li, Zhong Guo Yao Xue 6 (1997) 57–64.
[12] Z.H. Cheng, Y.P. Lou, H.J. Dai, F. Xue, Shang Hai Yi Ke Da Xue Xue Bao 14 (1987) 25–29.
[13] Z.T. Dong, W.D. Jiang, Acta Pharm Sinica. 17 (1982) 226–228.
[14] L.H. Tang, X.M. Wang, D.Q. Liang, Zhong Guo Bing Li Sheng Li Za Zhi 5 (1989) 65–69.
[15] Y.H. Chen, G.H. Du, J.T. Zhang, Zhong Guo Xin Yao Za Zhi 21 (2000) 463–466.
[16] B.L. Zhao, W. Jiang, Y. Zhao, J.W. Hou, W.J. Xin, Biochem. Mol. Biol. Int. 38 (1996) 1171–1182.
[17] The Pharmacopoeia Commission of PRC, Pharmacopoeia of the People’s Republic of China Version 2000, vol. 1, Beijing Chemical Industry Press, 2000.
[18] The Pharmacopoeia Commission of PRC, Pharmacopoeia of the People’s Republic of China Version 2005, vol. 1, Beijing Chemical Industry Press, 2005.
[19] Z.H. Shi, J.T. He, T.T. Yao, W.B. Chang, M.P. Zhao, J. Pharm. Biomed. Anal. 37 (2005) 481–486.
[20] P. Hu, G.A. Luo, Z.Z. Zhao, Z.H. Jiang, Chem. Pharm. Bull. 53 (2005) 481–486.
[21] H. Zhang, C. Yu, J.Y. Jia, S.W. Leung, Y.L. Siow, R.Y. Man, D.Y. Zhu, Acta Pharm. Sinica 23 (2002) 1163–1168.
[22] D. Yuan, Y. Pan, W. Fu, T. Makino, Y. Kano, Chem. Pharm. Bull. 53 (2005) 508–514.
[23] http://www.tasly.com.