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ORIGINAL ARTICLE

Simultaneous Determination of Eight Phenolic Acids, Five Saponins and Four Tanshinones for Quality Control of Compound Preparations Containing Danshen-Sanqi Herb-pair by HPLC-DAD

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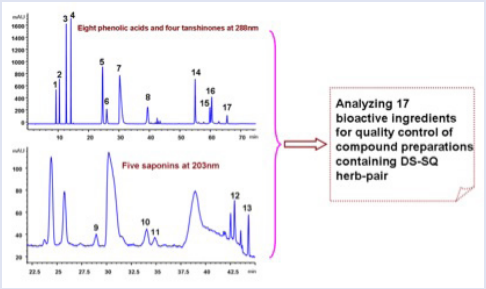
ABSTRACT

Background: The herb-pair, *Salviaemiltiorrhizae* (Danshen, DS) and *Panaxnotoginseng* (Sanqi,SQ), often occurs in traditional Chinese medicine prescriptions used for the treatment of cardiovascular diseases in clinics in Asian areas. Many commercial preparations containing the DS-SQ herb-pair were produced by various manufactures with the different production process. The raw materials were from different sources, which raised a challenge to control the quality of the herb-pair medicines. **Objective:** In this paper, a high-performance liquid chromatography (HPLC) method was developed to simultaneously determine seventeen bioactive components, including 8 phenolic acids, 4 tanshinones, and 5 saponins, for quality control of compound preparations containing DS-SQ herb-pair. The chromatographic separation was studied on an Ultimate™ XB-C_{18} column (150 mm × 4.6 mmi.d., 3.5 μm) with a mobile phase composed of 0.5% aqueous acetic acid and acetonitrile using a gradient elution in 70 min. **Results:** The optimum detection wavelength was set at 288 nm for phenolic acids and tanshinones, and 203 nm for saponins. The method was validated sufficiently by examining the precision, recoveries, linearity, range, LOD and LOQ, and was successfully applied to quantify the seventeen compounds in five commercial preparations containing DS-SQ herb-pair. **Conclusions:** It is the first time to report the rapid and simultaneous analysis of the seventeen compounds with the base-line separation of peaks for ginsenoside Rg_1 and Re in 70 min by routine HPLC. This HPLC method could be considered as good quality criteria to control the quality of preparations containing DS-SQ herb-pair.

Key words: Compound preparations, danshen-sanqi herb-pair, high-performance liquid chromatography, quality control

SUMMARY

- An HPLC method was originally developed to simultaneously quantify 8 phenolic acids, 4 tanshinones and 5 saponins in DS-SQ herb-pair preparations.
- The rapid and simultaneous analysis of the 17 compounds with the base-line separation of peaks for ginsenoside Rg_1 and Re within 70 min was achieved for the first time by routine HPLC.
- The presented method was successfully applied to the quality control of five compound preparations containing DS-SQ herb-pair.
- Additionally, it found that the favorable dosage forms for prescriptions containing DS-SQ herb-pair could be solid preparations.



Abbreviations used: DS: *Salviae miltiorrhizae*; SQ: *Panaxnotoginseng*; HPLC: high-performance liquid chromatography; DAD: diode array detector; LOD: limit of detection; LOQ: limit of quantification; TCMs: Traditional Chinese medicines; GDDP: Guanxin Danshen dripping pills; FDDP: Fufang Danshen dripping pills; FDT: Fufang Danshen tablets; FDC: Fufang Danshen capsules; GP: Guanxin pills

Key Messages:

The HPLC-DAD analysis successfully fulfilled the simultaneous determination of 17 compounds (including three types of authentic bioactive components, 8 phenolic acids, 4 tanshinones, and 5 saponins) in DS-SQ herb-pair within 70 min with the routine HPLC for the first time. The results also demonstrated that solid preparations could be the favorable dosage forms for those prescriptions containing DS-SQ herb-pair due to the instability of saponins from SQ, when the components of DS and SQ were coexisting in solution. The study provides a promising tool for quality control of the preparations containing the DS-SQ herb-pair.

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INTRODUCTION

The traditional Chinese medicines (TCMs) have been attracting more and more attention due to the treatment of a wide variety of ailments successfully with minimum side effects in many diseases.[1,2] Their remedial mechanisms are still not fully understood, but multiple ingredients belonging to different structural classes and possessing different mechanisms of action seem to be responsible for the therapeutic function of TCMs.[3] Chinese herbal formulae, consisting of several herbs in proportion, usually contain hundreds of different constituents. The simultaneous determination of different kinds of components in a Chinese herbal formula is significant to disclose the secret underlying their effectiveness and to enhance products quality control.

The herb-pair, consisting of *Salviae miltiorrhizae* (Danshen, DS) and *Panaxnotoginseng* (Sanqi, SQ), has been used to improve coronary or

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cerebral circulation in China as well as in Western countries for several decades.[4] Some compound preparations containing the DS-SQ herb-pair, such as Guanxin Danshen dripping pills (GDDP), Fufang Danshen dripping pills (FDDP), Fufang Danshen tablets (FDT), Fufang Danshen capsules (FDC), and Guanxin pills (GP) are commercially available and have been widely used for the treatment of coronary heart disease and angina pectoris,[5] viral myocarditis,[6] and silent myocardial ischemia[7] in clinics. These preparations are mainly prepared from the extract mixtures of Radix *Salvia miltiorrrhiza* and/or *Panaxnotoginseng*. There are three types of components in the preparations, including phenolic acids, tanshinones and saponins, which were related to the therapeutic efficacy of anti-cardiovascular/cerebrovascular diseases. The phenolic acids and tanshinones, such as danshensu, protocatechuic acid, protocatechuicaldehyde, caffeic acid, rosmarinic acid, lithospermic acid, salvianolic acids A and B,dihydrotanshinone I, cryptotanshinone, tanshinone I and tanshinone IIA have shown the effects of neuroprotection,[8-10] anti-platelet aggregation,[11-13] anti-thrombosis,[14] anti-arrhythmia,[15] anti-oxidation[16] or protection of the myocardium against ischemia.[17-20] Meanwhile, saponins from Sanqi, such as notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, and Rd could protect myocardium and cerebral tissues against ischemia.[21-25] An accurate and simple method for determining as many the above-mentioned bioactive components as possible becomes essential for understanding the therapeutic efficacy and quality control of the preparations containing the herb-pair.

Till now, a number of assays have been developed for determination of those bioactive components, which are above-mentioned, in some compound preparations containing DS-SQ herb-pair. For example, HPLC-UV or DAD,[4,26-29] HPLC-ELSD,[4] LC-MS,[30] fourier transform near infrared spectroscopy (FT-NIR)[31] and micro emulsion electro kinetic chromatography (MEEKC)[32] have been used to determine the phenolic acids, tanshinones or/and saponins in herbal preparations containing the herb-pair. However, except for HPLC–UV or DAD, the instruments used in other methods are relatively expensive and not routine, or may be unavailable in every laboratory.[4] Meanwhile, most of the reported quantitative methods referred to one or two types of components from only one comprising herb (Danshen or Sanqi),[29, 33-39] or determined a few components, without comprehensively considering the other authentic bioactive components as the marker ones.[26-39] Moreover, when giving overall consideration of the three types of components (phenolic acids, tanshinones, and saponins) in compound preparations containing DS-SQ herb-pair, it was difficult to fulfill the base-line separation between ginsenosides Rg_1 and Re (two of the main bioactive saponins in DS-SQ herb-pair) by HPLC, and most of the methods required a long chromatographic process (beyond 70 min).[4,39] For instance, an improved HPLC method with DAD and ELSD detectors had been reported for simultaneous determination of 4 phenolic acids, 4 saponins, and 4 tanshinones in 90 min.[4] With this method, 12 bioactive compounds, including danshensu, protocatechuic aldehyde, rosmarinic acid and salvianolic acid B, notoginsenoside R_1, ginsenosides Rg_1, Rb_1 and Rd,dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone IIA were successfully quantified in FDDP. The authentic bioactive phenolic acids, such as protocatechuic acid, caffeic acid, lithospermic acid, salvianolic acid A and ginsenoside Re were not quantified in the preparations. In addition, a few predominant works have been reported by using LC-MS.[40] and MEEKC[32] For example, Lai *et al.*[40] developed a mobile-phase compensation (MPC) method to overcome the ion-ization variance caused by mobile phase composition in HPLC–ESI-MS analyses for the relative quantification of multi-components in complex mixture, and successfully used for relative quantification of the minor asnqisaponins by their detected peak areas divided by that of ginsenoside Rd. The method provides the possibility on obtaining the normalized sharable data in different laboratories.

In this study, an HPLC-DAD method was proposed and validated to determine as many authentic bioactive compounds as possible in the preparations containing DS-SQ herb-pair. Owning to the simple, reliable and relatively rapid (below 70 min per chromatographic analysis) properties, it was applied to the quality control of 5 compound preparations containing DS-SQ herb-pair, that is GDDP, FDDP, FDT, FDC, and GP through simultaneous determination of three types of authentic bioactive components (the structures were shown in Figure 1, including eight majorphenolic acids, namely danshensu (1), protocatechuic acid (2), protocatechuic aldehyde (3), caffeic acid (4), rosmarinic acid (5), lithospermic acid (6), salvianolic acid B (7), and salvianolic acid A (8); five major saponins, namely notoginsenoside R_1 (9), ginsenosideRg_1 (10), ginsenoside Re (11), ginsenoside Rb_1(12), and ginsenoside Rd (13); and four major tanshinones, namely dihydrotanshinoneI (14), cryptotanshinone (15), tanshinone I (16), and tanshinone IIA (17).

Materials and Methods

Chemicals and materials

Reference compounds, sodium danshensu, protocatechuic acid, protocatechuicaldehyde, caffeic acid, rosmarinic acid, lithospermic acid, salvianolic acid B, salvianolic acid A, notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1, and Rd, dihydrotanshinone I, cryptotanshinone, tanshinone Iand tanshinone IIA were purchased from Shanghai Ronghe Medicine Technology Development Co. Ltd. (Shanghai, China). Guanxin Danshen dripping pills (GDDP, batch no.YR06524,YR06904 and YR06905),Fufang Danshen dripping pills (FDDP, batch no. 140609 and 140623), Fufang Danshen tablets (FDT, batch no.120901110), Fufang Danshen capsule (FDC, batch no.1400801), and Guanxin pills (GP, batch no.20130103 and 20130704) were purchased from local drug stores (Fuzhou, China).

HPLC grade acetonitrile (MerkKGaA, Darmstadt, Germany) was used for the HPLC analysis. Double distilled water for HPLC analysis was prepared in our lab. Chromatographic grade methanol was purchased from Xilong Chemical Co. Ltd., (Guangdong, China). Glacial acetic acid was a product of Shanghai Jingchun Chemical Reagent Co. Ltd. (Shanghai, China).

Apparatus and chromatographic conditions optimization

The analyses were performed using an Agilent-1260 series HPLC instrument (Agilent Technologies, USA) equipped with a low pressure quaternionic pump, an auto-sampler, a column compartment, and diode-array detection (DAD).

Four Ultimate™ XB-C_{18} columns (Welch Materials, Inc., Ellicott, MD, USA), (A) 50 mm × 4.6 mmi.d. 3.5 μm, (B) 100 mm × 4.6 mmi.d., 3.5 μm, (C) 150 mm × 4.6 mmi.d. 3.5 μm, and (D) 250 mm × 4.6 mmi.d. 5 μm were tested with the flow rates of 0.8, 0.8, 0.8 and 1 ml/min, respectively for chromatographic condition optimization. Taking water–acetic acid (99.5:0.5, v/v) and acetonitrile–acetic acid (99.5:0.5, v/v) as mobile phases A and B, respectively, the gradient conditions were optimized for the four columns. Meanwhile, the ultra-violet absorption spectrum of each chromatographic peak was recorded by the DAD detection for selecting the suitable detection wavelengths for determining phenolic acids, tanshinones, and saponins.

XB-C_{18} column, 150 mm × 4.6 mmi.d.3.5 μm, (Welch Materials, Inc., Ellicott, MD, USA) for the sample analysis. The mobile phase consisted of water-acetic acid (99.5:0.5, v/v) and acetonitrile–acetic acid (99.5:0.5, v/v). An optimized gradient program was carried out as follows: 0-6 min, start with 2% B, then linearly increase to 10% B; 6-10 min, linearly

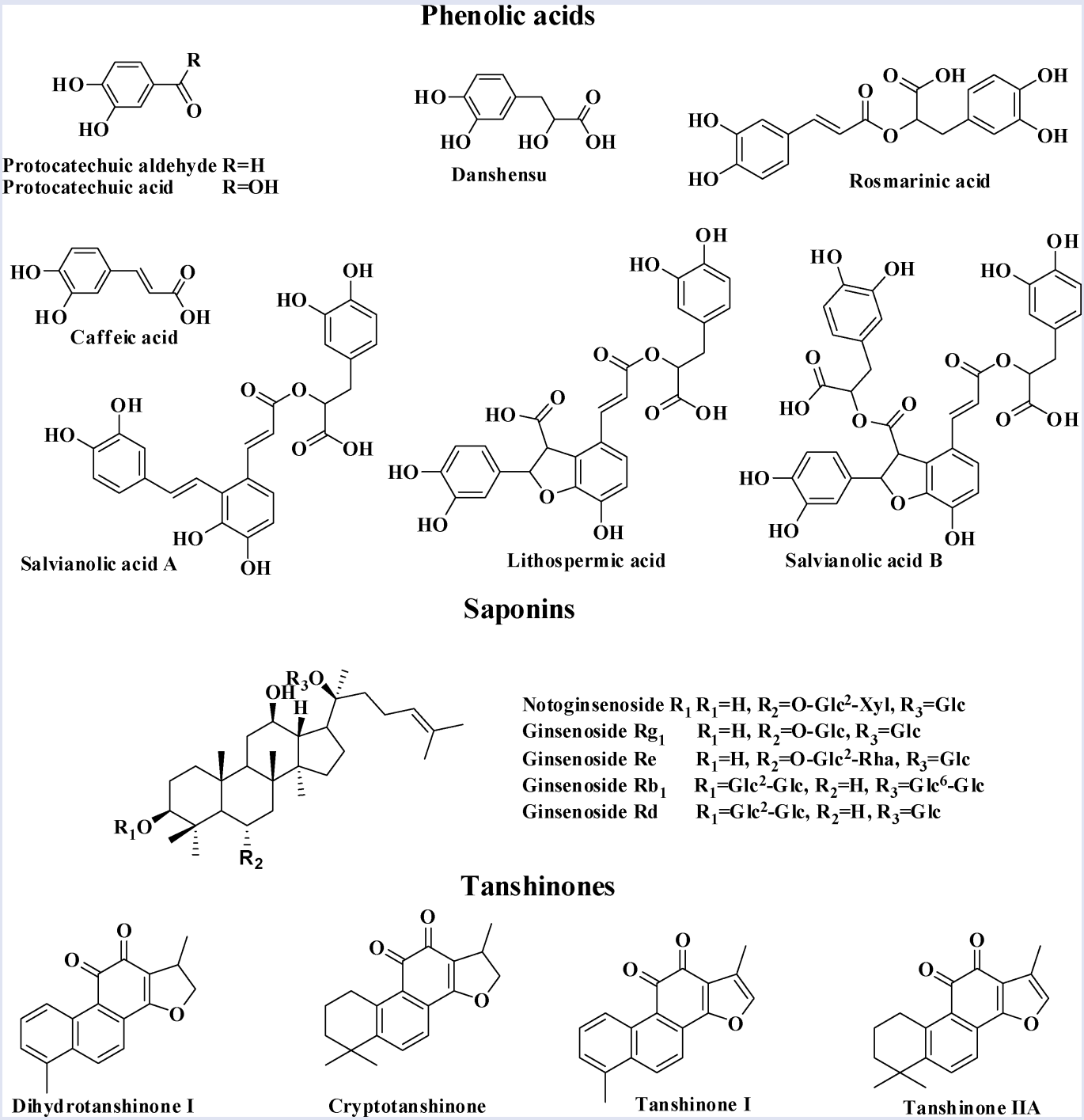

Figure 1: Chemical structures of the 17 compounds.

increase to 19% B; 10-16 min, linearly increase to 21.2% B; 16-35 min, linearly increase to 23% B; 35-–40 min, linearly increase to 45% B; 40-45 min, linearly increase to 50% B; 45-65 min, linearly increase to 80% B; 65-66 min, linearly decrease to 2% B; then 2% B at 66-70 min, giving a total run time of 70 min. The flow rate was 0.8 mL/min, and the column temperature was set at 30°C. The detection wavelength was set at 288 nm for monitoring phenolic acids and tanshinones, and 203 nm for saponins.

Sample preparation

The extraction solvent was optimized with GDDP (batch no. YR05627) as a carrier. 50%, 70%, 90% and 100% methanol (v/v) were tested as the extraction solvent. GDDP was ground into fine powder. An aliquot of 1 g of the powder was transferred into a 10 mL-volumetric flask and ultrasonically extracted with 10 mL of 50%, 70%, 90% or 100% methanol for 30 min for one time. The homologous extraction solvent (50%, 70%,

90% or 100% methanol) was then added for compensating the volume lost during the ultrasonic process. The supernatant was filtered through a 0.45 μm membrane, and 8 mL of the solution was injected for HPLC-DAD analysis. The best extraction efficiency was obtained by using 100% methanol.

To optimize extraction frequency, after the ultrasonic extraction of 1g powder sample with 10 mL methanol for 30 min, the extract was filtered and the residue was extracted repeatedly with 10 mL methanol for another 30 min. The second extract was then injected into HPLC for analysis after filtration. As a result, the selected extraction frequency was one time.

Ultimately, for sample analysis, GDDP, FDDP, FDT, FDC, or GP were treated with the conditions above-optimized. 8 mL of each sample solution was injected for HPLC-DAD analysis.

Method validation

Calibration curves, limits of detection and quantification

The standard stock solutions of 8phenolic acids, 5 saponins and 4 tanshinones, were respectively prepared in volumetric flasks with methanol, methanol, and methanol-chloroform (2:3, v/v). Before analysis, 0.3 mL of each kind of standard stock solution and 0.1 mL of methanol were transferred to a 1 mL-volumetric flask to make the mixture solution of the 17 reference compounds, and the concentration of each compound was 0.900 mg/mL (1), 0.330 mg/mL (2), 0.300 mg/mL (3), 0.345 mg/mL (4), 0.795 mg/mL (5), 0.300 mg/mL (6), 3.030 mg/mL (7), 0.345 mg/mL (8), 1.530 mg/mL (9), 1.515 mg/mL (10), 1.530 mg/mL (11) 3.090 mg/mL (12), 1.800 mg/mL (13), 0.360 mg/mL (14),0.330 mg/mL (15), 0.300 mg/mL (16), and 0.360 mg/mL (17), respectively. Then, the mixed stock solution was further diluted with methanol to obtain 13 different concentration ranges including 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, and 1/4096 of the original concentration. All the solutions were stored in a refrigerator (4°C).The calibration curve for each compound was established by plotting the peak areas versus the concentration. The limits of detection (LOD) for each component were determined at a signal-to-noise ratio of 3, while the limits of quantification (LOQ) were evaluated at signal-to-noise ratio of 10.

Precision, repeatability and stability

The intra-day precision was tested by assaying the low, middle and high concentrations of mixed standard solution within 1 day in four times, and the inter-day precision was determined three times in 3 consecutive days. The relative error (RE) and relative standard deviation (R.S.D.) were taken as the measures of precision. To evaluate the repeatability of the developed assay, six samples from the same batch of GDDP (batch no. YR28895), were treated according to the sample preparation procedure as described in the Section of *Sample preparation* and analyzed with the established method. The R.S.D. was taken as the measure of repeatability. The stability was confirmed with a sample of GDDP treated with the preparation method as described in the Section of *Sample preparation* at room temperature and analyzed at 0, 2, 4, 8, 10, 24, 36, and 48 h. The RE of the determined concentration at each time point compared to the nominal concentration was taken as the measure of stability.

Recovery

1 g of nine powder samples of GDDP (batch no. YR28894 and YR28893) was respectively weighed and spiked with low, middle and high known amounts of reference compounds, then prepared as described in the Section of *Sample preparation* and analyzed with the developed HPLC method. The quantity of each compound was subsequently calculated from the corresponding calibration curve. Recovery (%) was calculated by the equation $(amount_{determined} - amount_{original})/amount_{spiked} \times 100$.[4]

Results and Discussion

Optimization of sample pretreatment

To get high extraction efficiency, extraction solvent and extraction frequency were optimized with GDDP (batch no. YR05627) as a carrier. 50%, 70%, 90% and 100% methanol were tested as the extraction solvent. As shown in Figure 2, the best extraction efficiency was obtained by using 100% methanol, since there were as many as chromatographic peak areas of the 17 components, which reached the highest values. Therefore, 100% methanol was selected as the extraction solvent. To investigate extraction frequency, after the ultrasonic extraction of powder samples with 10 mL extraction solvent for 30 min, the extract was filtered and the residue was extracted repeatedly with 10 mL extraction solvent for another 30 min. The second extract was then injected into HPLC for analysis after filtration. However, there were no essentially peaks in the chromatogram. Therefore, the selected extraction frequency was one time.

Optimization of chromatographic conditions

We optimized the separation conditions including the column specification, elution gradient and detection wavelength in this study. The four Ultimate™ XB-C_{18} columns, (A) 50 mm × 4.6 mmi.d. 3.5 μm, (B) 100 mm × 4.6 mmi.d., 3.5 μm, (C) 150 mm × 4.6 mmi.d. 3.5 μm, and (D) 250 mm × 4.6 mmi.d. 5 μm were tested. The results showed that except for Rg_1 and Re, or cryptotanshinone and tanshinone I, the base-line separation for the most compounds studied could be obtained with the four columns by HPLC. Meanwhile, only the base-line separation for all the 17 compounds studied could be obtained with the column C. Therefore, the column C (150 mm × 4.6 mm, i.d. 3.5 μm) was selected at the subsequent study. And also, it is the first time to report the rapid and simultaneous analysis of the seventeen compounds accompanying with the base-line separation between ginsenoside Rg_1 and Re in 70 min by routine HPLC.

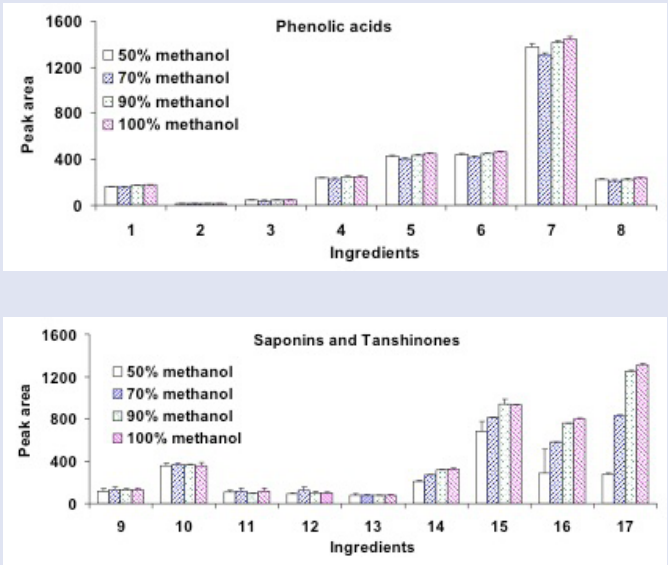

Figure 2: Histograms of peak areas for 17 compounds in GDDP, including danshensu (1), protocatechuic acid (2), protocatechuic aldehyde (3), caffeic acid (4), rosmarinic acid (5), lithospermic acid (6), salvianolic acid B (7), salvianolic acid A (8), notoginsenoside R_1 (9), ginsenosides Rg_1 (10), Re (11), Rb_1 (12), and Rd (13), dihydrotanshinone I (14), cryptotanshinone (15), tanshinone I (16) and tanshinone IIA (17), with 50%, 70%, 90% and 100% methanol as extraction solvent by HPLC (mean ± SD, *n*=3).

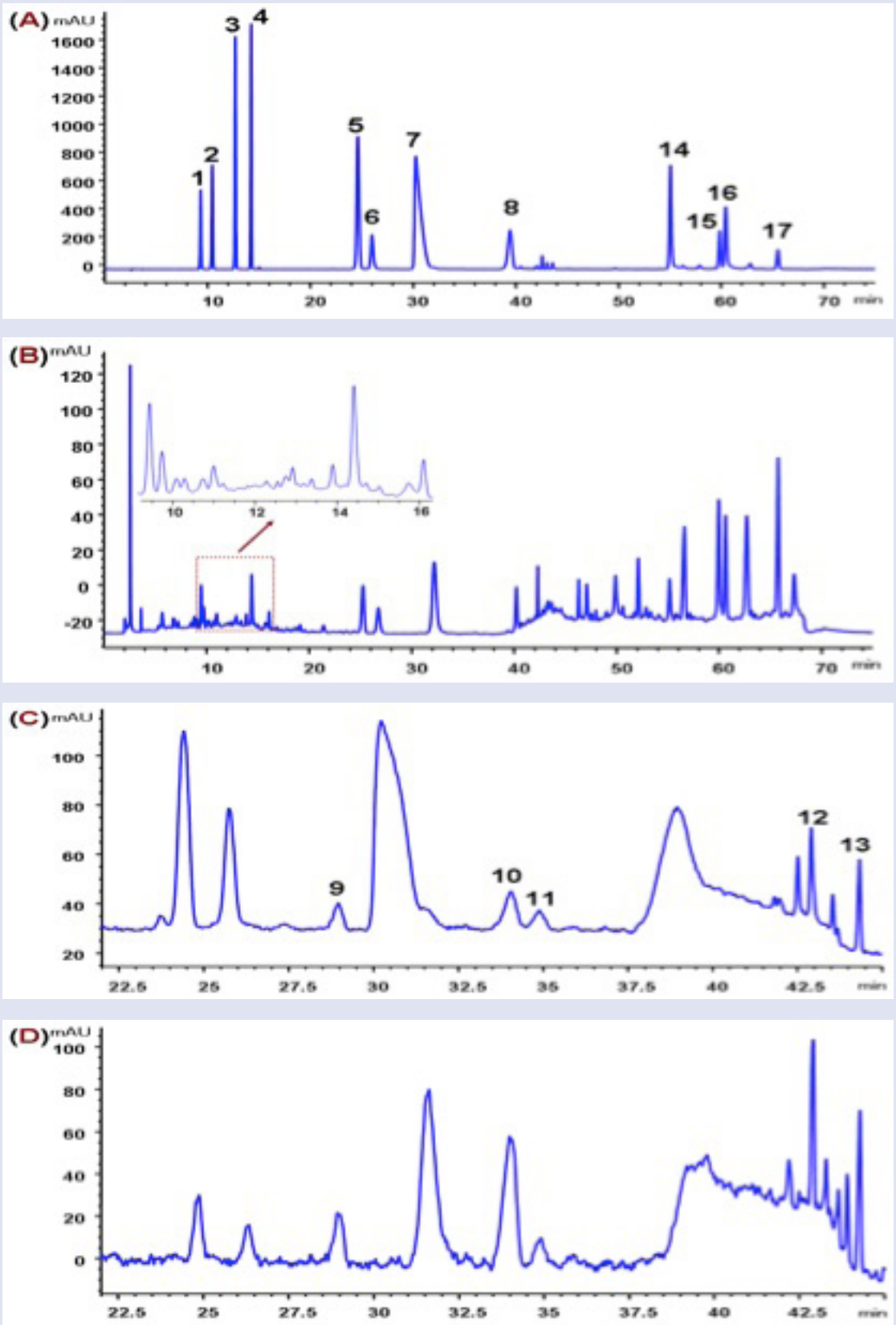

Figure 3: Typical HPLC-DAD chromatograms of 17 standard references (A) and GDDP sample (B) at 288 nm, and standard references (C) and GDDP sample (D) at 203 nm. Peaks (1) sodium danshensu, (2) protocatechuic acid, (3) protocatechuic aldehyde, (4) caffeic acid, (5) rosmarinic acid, (6) lithospermic acid, (7) salvianolic acid B, (8) salvianolic acid A, (9) notoginsenoside R_1, (10) ginsenoside Rg_1, (11) ginsenoside Re, (12) ginsenoside Rb_1, (13) ginsenoside Rd, (14) dihydrotanshinone I, (15) cryptotanshinone, (16) tanshinone I and (17) tanshinone IIA.

According to the UV maximal absorption of the 8 phenolic acids and 4 tanshinones, the chromatograms for the components in Danshen were recorded at 288 nm. Meanwhile, the detection at 203 nm was utilized for monitoring the 5 saponins in Sanqi, consistent with our previous study (Yao *et al*. 2011). The attribution of each peak in samples was confirmed by contrasting retention time and UV spectrum of each peak with that of reference compound. Representative HPLC–DAD chromatograms of the 17 reference compounds, GDDP sample were shown in Figure 3.

Method validation results

Table 1 lists calibration curve, linear range, R^2, LOD, and LOQ of each compound. All the compounds showed a good linearity (R^2> 0.9944) in the relatively wide concentration range. LOD was in the range of 0.56–5.92μg/ml, 5.92–12.08μg/ml, 0.59–0.71μg/ml for phenolic acids, saponins and tanshinones, respectively; and LOQ was in the range of 1.11–11.84 μg/ml, 11.84–24.16 μg/ml, 1.17–1.41μg/ml for phenolic acids, saponins and tanshinones, respectively.

Table 2 shows the results of intra-day and inter-day precision of the 17 components. The overall R.S.D. of the intra-day precision was 0.24–6.36%. The overall R.S.D. of the inter-day precision was 1.30-7.10%.

Table 3 lists repeatability and stability of each compound. The overall R.S.D. of the repeatability was 0.90–11.53%. The overall absolute value of RE for the stability was 0.14–11.87% within 10 h. However, as shown in Table 3, the absolute value of RE for the stability of saponins was much more negative than –40% beyond 12h, and the descent was more apparent with extending the storage period of sample solution from 12h to 48h. The results suggested that saponins from SQ were instable when the components from DS and SQ coexisted in solution, and especially, it was better to perform the HPLC analysis within 10h after completing the preparation of the sample solution. In addition, it also suggested that solid preparations could be the favorable dosage forms for those prescriptions containing DS-SQ herb-pair due to the instability of saponins of SQ when coexisting with the components of DS in solution.

As shown in Table 4, the recoveries for the 17 compounds were favorable (87.41-107.35%). The results of the recovery test indicated that the method developed was available for determination of the 17 bioactive components in preparations containing the DS-SQ herb-pair.

Sample analysis

The developed method was applied to simultaneously quantify the 8 phenolic acids, 5 saponins, and 4 tanshinones in GDDP, FDDP, FDT, FDC, and GP. The results [Table 5] showed that the total phenolic acids contents in these preparations ranged from 1.44 to 20.11 mg/g, the saponins varied from 1.33 to 26.86mg/g except for GP, and the tanshinones ranged from 0.64 to 4.91mg/g, among different manufacturers/or batches. The total contents of phenolic acids in FDDP, FDT and FDC samples were similar and about 10 time higher than those in GP and GDDP samples; among the 8 phenolic acids, the content of salvianolic acid Bin FDC sample was highest than those in all the other samples. The total content of saponins in FDC sample was lower than those in GDDP, FDDP and FDT samples, while the total content of saponins in GP sample was very difficultly detected by the presented HPLC method, possibly owing to the preparation process involving a distinctive procedure "preparing water pills" in GP production. Meanwhile, it could also be found that among the four tanshinones in all the samples studied, the content of tanshinone II A was the highest. Summarily, the contents of the three types of compounds varied markedly among DS-SQ herb-pair preparations with different brand. The reason might be due to different proportion of DS to SQ, different preparation process or the quality inconsistency of the crude materials used to produce the preparations.

Table 1: Detection wavelength, calibration curves, linear range, LOD, and LOQ of the 17 components

Analytes	Detection wavelength (nm)	Calibration curves[a]	Linear range (μg/mL)	R^2	LOD (μg/mL)	LOQ (μg/mL)
Danshensu	288	$y = 4.5387x - 1.2456$	3.52-900.00	0.9998	1.76	3.52
Protocatechuic acid	288	$y = 15.503x - 21.335$	1.29-330.00	0.9997	0.65	1.29
Protocatechualdehyde	288	$y = 35.142x - 5.5682$	1.11-300.00	0.9998	0.56	1.11
Caffeic acid	288	$y = 31.722x - 22.766$	1.35-345.00	0.9998	0.68	1.35
Rosmarinic acid	288	$y = 21.097x - 101.85$	3.11-795.00	0.9997	1.56	3.11
Lithospermic acid	288	$y = 10.808x - 24.265$	1.11-300.00	0.9992	0.56	1.11
Salvianolic acid B	288	$y = 11.345x - 115.46$	11.84-3030.00	0.9994	5.92	11.84
Salvianolic acid A	288	$y = 14.793x - 50.148$	1.35-345.00	0.9994	0.68	1.35
Notoginsenoside R_1	203	$y = 1.1736x + 4.6435$	24.16-1530.00	0.9978	12.08	24.16
Ginsenosides Rg_1	203	$y = 1.0126x + 157.01$	11.84-3030.00	0.9990	5.92	11.84
Ginsenosides Re	203	$y = 0.5837x + 130.54$	11.95-1530.00	0.9969	5.98	11.95
Ginsenosides Rb_1	203	$y = 0.2788x + 30.922$	12.07-3090.00	0.9988	6.04	12.07
GinsenosidesRd	203	$y = 0.2041x + 12.789$	14.07-1800.00	0.9944	7.03	14.07
Dihydrotanshinone I	288	$y = 36.902x - 33.614$	1.41-360.00	0.9998	0.71	1.41
Cryptotanshinone	288	$y = 13.662x + 4.9437$	1.29-330.00	0.9998	0.65	1.29
Tanshinone I	288	$y = 19.543x + 10.609$	1.17-300.00	0.9998	0.59	1.17
Tanshinone IIA	288	$y = 7.4264x - 4.9166$	1.41-360.00	0.9998	0.71	1.41

a y: peak area of analyte; x: concerntration of analyte (μg/mL).

Table 2: Intra-day and inter-day precision of the 17 components

Analytes	Concentration spiked (μg/mL)	Intra-day (n = 4)		Inter-day (n = 3)	
		Detected (μg/mL)	R.S.D. (%)	Detected (μg/mL)	R.S.D. (%)
Danshensu	900.00	979 ± 10.87	1.11	1026.90 ± 42.44	4.13
	450.00	461.30 ± 2.00	0.43	481.70 ± 20.20	4.19
	225.00	221.06 ± 3.06	1.39	233.29 ± 8.99	3.85
Protocatechuic acid	330.00	352.33 ± 3.93	1.11	374.32 ± 17.23	4.60
	165.00	165.24 ± 0.57	0.35	172.49 ± 7.37	4.28
	82.50	79.81 ± 0.96	1.21	84.24 ± 3.43	4.08
Protocatechualdehyde	300.00	322.97 ± 3.27	1.01	344.60 ± 18.16	5.27
	150.00	151.65 ± 0.43	0.28	158.36 ± 6.96	4.39
	75.00	72.64 ± 1.02	1.41	76.67 ± 3.03	3.95
Caffeic acid	345.00	365.70 ± 3.79	1.04	394.30 ± 25.71	6.52
	172.50	177.22 ± 0.58	0.33	185.02 ± 7.87	4.25
	86.25	84.75 ± 1.16	1.36	88.93 ± 3.15	3.54
Rosmarinic acid	795.00	858.05 ± 8.46	0.99	922.20 ± 52.52	5.69
	397.50	402.05 ± 1.71	0.43	420.49 ± 21.20	5.04
	198.75	191.08 ± 3.76	1.97	201.75 ± 6.87	3.40
Lithospermic acid	300.00	319.53 ± 2.45	0.77	346.20 ± 24.58	7.10
	150.00	148.76 ± 3.16	2.12	154.94 ± 9.55	6.16
	75.00	70.31 ± 2.61	3.72	73.74 ± 1.85	2.50
Salvianolic acid B	3030.00	3353.98 ± 37.79	1.13	3582.96 ± 181.19	5.06
	1515.00	1565.07 ± 3.69	0.24	1620.55 ± 66.51	4.10
	757.50	740.14 ± 13.04	1.76	773.87 ± 21.65	2.80
Salvianolic acid A	345.00	370.95 ± 5.00	1.35	393.31 ± 21.54	5.48
	172.50	152.36 ± 3.20	2.10	150.71 ± 1.96	1.30
	86.25	68.14 ± 1.62	2.38	66.65 ± 2.72	4.07
Notoginsenoside R_1	765.00	744.06 ± 29.51	3.97	729.29 ± 29.91	4.10
	382.50	364.49 ± 22.03	6.04	375.76 ± 22.43	5.97
	191.25	179.17 ± 10.05	5.61	174.36 ± 16.60	9.52
Ginsenosides Rg_1	1515.00	1533.33 ± 78.53	5.12	1501.77 ± 51.95	3.46
	757.50	735.29 ± 41.83	5.69	746.15 ± 33.18	4.45
	378.75	348.37 ± 16.75	4.81	355.41 ± 12.54	3.53
Ginsenosides Re	1530.00	1561.50 ± 97.49	6.24	1597.15 ± 104.13	6.52
	765.00	751.03 ± 24.77	3.30	744.08 ± 50.09	6.73
	382.50	389.96 ± 16.15	4.14	377.62 ± 19.17	5.08
Ginsenosides Rb_1	3090.00	3147.54 ± 96.53	3.07	1581.34 ± 120.53	7.62
	1545.00	1530.88 ± 97.33	6.36	1572.47 ± 101.56	6.46
	772.50	752.49 ± 28.30	3.76	768.39 ± 37.93	4.94

continued

Analytes	Concentration spiked (μg/mL)	Intra-day (n = 4)		Inter-day (n = 3)	
		Detected (μg/mL)	R.S.D. (%)	Detected (μg/mL)	R.S.D. (%)
Ginsenosides Rd	**900.00**	**916.71 ± 55.80**	**6.09**	**885.52 ± 40.03**	**4.52**
	450.00	447.99 ± 27.65	6.17	467.33 ± 21.42	4.58
	225.00	217.37 ± 13.49	6.21	215.06 ± 13.95	6.49
Dihydrotanshinone I	360.00	388.29 ± 5.90	1.52	402.66 ± 6.87	1.71
	180.00	179.90 ± 0.54	0.30	187.95 ± 8.69	4.62
	90.00	87.32 ± 1.53	1.75	92.08 ± 3.24	3.52
Cryptotanshinone	330.00	366.39 ± 4.34	1.18	394.40 ± 20.33	5.15
	165.00	169.71 ± 1.27	0.75	178.17 ± 8.52	4.78
	82.50	82.33 ± 1.74	2.12	86.28 ± 2.51	2.91
Tanshinone I	300.00	331.06 ± 3.30	1.00	353.25 ± 15.45	4.37
	150.00	152.12 ± 0.71	0.47	158.85 ± 8.69	4.28
	75.00	73.27 ± 1.18	1.61	76.93 ± 2.51	3.26
Tanshinone IIA	360.00	392.29 ± 3.54	0.90	415.50 ± 21.72	5.23
	180.00	182.09 ± 0.69	0.38	189.36 ± 7.41	3.91
	90.00	88.68 ± 1.13	1.28	93.053 ± 3.31	3.55

Table 3: Repeatability and stability of the 17 components (n = 6)

Analytes	Repeatability (R.S.D., %)	Stability [a] (RE, %)						
		Nominal (μg/g)	2 h	4 h	8 h	12 h	24 h	48 h
Danshensu	2.49	65.75	2.38	4.13	5.53	3.43	1.33	28.98
Protocatechuic acid	4.81	16.24	-0.63	0.22	2.75	3.18	9.09	-23.87
Protocatechualdehyde	2.18	8.78	-1.48	-0.51	1.76	-0.83	0.79	1.11
Caffeic acid	4.65	34.86	0.60	1.32	5.64	15.25	19.66	20.65
Rosmarinic acid	0.90	223.56	0.14	0.68	3.50	3.88	8.47	5.71
Lithospermic acid	1.97	117.09	0.97	0.29	3.07	1.57	5.02	7.34
Salvianolic acid B	1.86	738.52	-7.34	-7.33	-7.32	-7.29	-7.28	-7.28
Salvianolic acid A	2.50	55.99	-1.67	-1.67	-0.10	1.81	-2.01	-4.36
Notoginsenoside R_1	5.20	6348.21	-7.58	-11.87	-11.17	-66.45	-64.48	-77.89
Ginsenosides Rg_1	2.34	21940.68	1.74	0.89	1.90	-72.87	-77.32	-79.78
Ginsenosides Re	11.53	1644.72	8.39	3.07	-7.46	-82.33	-89.92	-95.12
Ginsenosides Rb_1	5.86	17753.96	-0.59	6.38	4.73	-71.66	-74.51	-90.12
Ginsenosides Rd	5.71	38173.60	7.13	1.56	6.88	-43.76	-55.96	-75.53
Dihydrotanshinone I	2.31	71.68	6.61	7.18	8.21	13.12	19.78	24.11
Cryptotanshinone	1.07	970.77	3.34	4.73	1.57	2.19	-6.81	-7.47
Tanshinone I	2.88	594.21	0.75	0.79	4.30	4.08	9.71	12.51
Tanshinone IIA	1.64	3040.96	0.63	0.78	3.88	3.45	9.19	9.95

Table 4: Recoveries of the 17 components

Analytes	Original mean (μg/g)	Spiked mean (μg/g)	Detected mean (μg/g)	Recovery mean (%)	R.S.D (%) (n = 3)
Danshensu	118.35	450.00	525.65	90.51	3.97
	120.13	300.00	417.78	99.22	1.80
	123.68	145.00	260.73	94.44	3.96
Protocatechuic acid	1.12	165.00	153.67	92.45	2.00
	1.97	110.00	111.92	99.96	2.14
	3.67	53.17	54.72	96.12	2.81
Protocatechualdehyde	4.03	150.00	149.25	96.81	0.50
	4.70	100.00	105.95	101.25	7.37
	6.03	48.33	56.92	105.32	0.82
Caffeic acid	62.40	142.50	192.98	91.64	1.52
	64.25	95.00	157.47	98.13	2.53
	67.95	45.92	110.82	93.56	5.54
Rosmarinic acid	168.98	382.50	505.46	87.97	1.08
	174.36	255.00	418.74	95.84	5.87
	185.12	123.25	298.42	91.94	2.39
Lithospermic acid	130.62	150.00	266.32	90.47	6.57
	133.81	100.00	230.58	96.77	5.01
	140.19	48.33	185.92	94.56	2.92
Salvianolic acid B	1191.70	1507.50	2511.84	87.57	1.85
	1175.21	1005.00	2162.38	98.23	2.86
	1142.24	485.75	1612.67	96.92	3.61
Salvianolic acid A	80.86	157.50	224.71	91.33	0.51
	79.85	105.00	186.22	101.30	2.49
	77.82	52.50	127.46	94.89	6.21
Notoginsenoside R_1	4761.51	10000.00	14294.51	95.33	3.50
	4833.65	5000.00	9603.10	95.39	1.52
	4977.95	2533.33	7341.01	93.28	0.46
Ginsenosides Rg_1	21239.63	25033.33	43762.74	89.97	2.23
	21220.55	15100.00	34931.44	90.80	6.29
	21182.40	8000.00	29049.61	98.34	4.09
Ginsenosides Re	5440.98	3100.00	8769.49	107.35	1.40
	5130.94	1566.67	6574.19	92.20	6.70
	4510.86	1066.67	5442.06	87.41	6.24
Ginsenosides Rb_1	13510.64	30000.00	42061.64	95.17	5.30
	13057.95	20033.33	32696.62	98.03	4.66
	12152.57	10033.33	21538.75	93.55	6.42
Ginsenosides Rd	5732.69	10000.00	15308.69	95.76	6.17
	5582.05	6000.00	11109.85	92.13	5.29
	5280.77	3066.67	8271.07	97.51	4.76
Dihydrotanshinone I	211.30	500.00	704.59	98.66	7.60
	208.13	400.00	628.00	104.97	4.59
	201.79	300.00	517.22	105.14	1.47
Cryptotanshinone	1204.08	1200.00	2362.49	96.53	3.54
	1193.89	1100.00	2249.33	95.95	7.08
	1173.52	1000.00	2144.53	97.10	10.88
Tanshinone I	572.52	510.00	1078.39	99.19	1.18
	549.14	408.00	965.86	102.14	3.87
	502.36	306.00	773.90	88.74	2.76
Tanshinone IIA	2790.99	1366.67	4158.69	100.59	9.04
	2815.59	1000.00	3754.95	93.94	3.88
	2864.80	900.00	3765.02	100.02	6.63

Table 5: Contents of the 17 components in GDDP, FDDP, FDT, FDC, and GP. (μg/g) (n = 3)

Analytes	GDDP						FDDP				FDT		FDC		GP			
	YR06524		YR06904		YR06905		140609		140623		120901110		1400801		20130103		20130704	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Danshensu[a]	287.55	0.28	159.21	0.22	313.74	0.33	7948.44	0.30	7504.02	0.15	1632.69	0.08	873.27	0.47	807.48	0.29	786.06	0.24
Protocatechuic acid	26.40	0.18	26.60	0.03	21.60	0.07	nd[b]		nd		26.90	0.01	29.80	0.72	35.60	0.08	45.90	0.08
Protocatechualdehyde	12.30	0.03	8.60	0.01	13.60	0.02	2987.10	0.03	3011.50	0.47	308.50	0.01	45.70	0.07	99.50	0.05	89.10	0.03
Caffeic acid	50.90	0.04	29.70	0.04	58.90	0.07	68.00	0.00	72.40	0.03	42.80	0.05	23.40	0.05	40.80	0.02	0.30	0.02
Rosmarinic acid	483.80	0.28	304.70	0.2	553.70	0.36	3595.60	0.21	4035.90	0.14	2276.30	0.03	2106.10	1.58	70.30	0.07	147.50	0.02
Lithospermic acid	378.21	0.20	201.66	0.23	427.98	0.33	344.53	0.01	315.45	0.04	315.45	0.04	1053.01	2.11	240.92	0.03	256.37	0.11
Salvianolic acid B	1045.00	0.32	628.50	0.15	1207.00	0.18	1048.50	0.09	1019.10	0.09	4659.60	0.06	15265.20	0.45	365.50	0.05	316.80	0.06
Salvianolic acid A	125.00	0.08	78.70	0.06	138.80	0.18	2786.90	0.89	2866.60	0.06	1534.00	0.07	714.00	2.41	463.80	0.04	343.10	0.07
Total	2409.16		1437.67		2735.32		18779.07		18824.97		10796.24		20110.48		2123.90		1985.13	
Notoginsenoside R_1	1221.00	0.38	1262.70	0.93	2338.40	4.93	5028.00	3.25	4682.80	0.62	1392.80	0.79	nd		nd		nd	
Ginsenosides Rg_1	6119.50	3.06	4392.20	2.07	4502.30	0.78	1435.40	2.77	2324.70	1.15	2803.70	1.35	nd		nd		nd	
Ginsenosides Re	238.06	0.44	284.07	0.58	175.25	0.13	nd		nd		339.58	0.67	nd		nd		nd	
Ginsenosides Rb_1	4040.30	1.84	2971.60	1.75	3922.90	4.47	835.00	2.01	828.00	2.01	3547.90	1.06	325.70	1.45	nd		nd	
Ginsenosides Rd	15146.80	1.92	12062.50	3.83	15925.80	2.93	2918.20	6.16	2950.10	3.99	7994.80	3.99	1008.90	1.92	nd		nd	
Total	26765.66		20973.07		26864.65		10216.60		10785.60		16078.78		1334.60		nd		nd	
Dihydrotanshinone I	56.60	0.02	25.90	0.05	55.30	0.04	nd		nd		256.70	0.02	272.80	0.01	101.40	0.05	25.40	0.01
Cryptotanshinone	665.00	0.58	606.20	0.23	741.50	0.14	4.30	0.02	13.70	0.05	1251.70	0.17	764.40	0.25	41.60	0.07	18.00	0.01
Tanshinone I	489.90	0.26	321.20	0.12	515.60	0.08	20.70	0.02	2.60	0.01	1328.30	0.08	1445.50	0.10	4.80	0.01	1.50	0.01
Tanshinone IIA	3212.17	2.31	2230.69	0.71	3598.91	0.65	466.11	0.75	539.40	1.54	4034.51	0.35	2510.47	0.36	606.57	0.33	603.55	0.85
Total	4423.67		3183.99		4911.31		491.11		555.70		6871.21		4993.17		754.37		648.45	

a Analyte referred to danshensu. b Not detecteda Analytereferred to danshensu.b Not detected

It is significant to determine as many bioactive components as possible for quality evaluation of these preparations containing DS-SQ herb-pair.

CONCLUSIONS

A simple, rapid and reliable HPLC-DAD method was developed for simultaneous determination of 8 phenolic acids, 4 tanshinones and 5 saponins. The method was successfully applied to quantify the 17 major components in 9 commercial samples of GDDP, FDDP, FDT, FDC, and GP. The results suggested that this HPLC method could be considered as good quality criteria to control the quality of preparations containing DS-SQ herb-pair. In addition, solid preparations could be the favorable dosage forms for those prescriptions containing DS-SQ herb-pair due to the instability of saponins from SQ when the components of DS and SQ coexist in solution.

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Conflicts of interest

There are no conflicts of interest.

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