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

Hong Yao¹, Xiaomei Huang¹, Shaoguang Li¹, Youjia Wu¹, Xinhua Lin¹, Peiying Shi²

¹Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, No.1 Xueyuan Road, Minhou County, Fuzhou, China
²Department of Traditional Chinese Medicine Resource and Bee products, Fujian Agriculture and Forestry University, No.15 Shangxiadian Road, Fuzhou, China

Submitted: 30-07-2015 Revised: 29-09-2015 Published: 06-01-2017

ABSTRACT

Background: The herb-pair, *Salviae miltiorrhizae* (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-C18 column (150 mm x 4.6 mm.i.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 separation 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 Rg1 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 Rg1 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.

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.

Correspondence:

Prof. Hong Yao,
Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, Fuzhou, China
E-mail: yauhong@126.com
DOI: 10.4103/0973-1296.197651

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.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.² 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.
cerebral circulation in China as well as in Western countries for several decades. 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, viral myocarditis, and silent myocardial ischemia in clinics. These preparations are mainly prepared from the extract mixtures of Radix Salvia miltiorrhiza and/or Panax notoginseng. 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, protocatechuic aldehyde, 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, anti-platelet aggregation, anti-thrombosis, anti-arrhythmia, or protection of the myocardium. Meanwhile, saponins from Sanqi, such as notoginsenoside R1, ginsenosides Rg1, Re, Rb1, and Rd could protect myocardium and cerebral tissues against ischemia. An accurate and simple method for determining as many as the above-mentioned bioactive components as possible became 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/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. Meanwhile, most of the reported quantitative methods referred to one or two types of components from only one comprising herb (Dansen or Sanqi),[21,22-29] or determined a few components, without comprehensively considering the other authentic bioactive components as the marker ones.[20-29] 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 Rg1 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).[4,30] 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, salvianolic acid B, notoginsenoside R1, ginsenosides Rg1, Rb1, 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 ansaquinapins 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 major phenolic 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 R1 (9), ginsenoside Rg1 (10), ginsenoside Re (11), ginsenoside Rb2 (12), and ginsenoside Rd (13); and four major tanshinones, namely dihydrotanshinone I (14), cryptotanshinone (15), tanshinone I (16), and tanshinone IIA (17).

MATERIALS AND METHODS

Chemicals and materials

Reference compounds, sodium danshensu, protocatechuic acid, protocatechuic aldehyde, caffeic acid, rosmarinic acid, lithospermic acid, salvianolic acid B, salvianolic acid A, notoginsenoside R1, ginsenosides Rg1, Rb1, and Rd, dihydrotanshinone I, cryptotanshinone, tanshinone I and 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. 1408081), and Guanxin pills (GP, batch no. 20130103 and 20130704) were purchased from local drug stores (Fuzhou, China). HPLC grade acetonitrile (MerckKGaA, 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-C18 columns (Welch Materials, Inc., Ellicott, MD, USA), (A) 50 mm × 4.6 mm i.d. 3.5 μm, (B) 100 mm × 4.6 mm i.d., 3.5 μm, (C) 150 mm × 4.6 mm i.d. 3.5 μm, and (D) 250 mm × 4.6 mm i.d. 5 μm were tested with the flow rates of 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-C18 column, 150 mm × 4.6 mm i.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 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 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.

**Figure 1:** Chemical structures of the 17 compounds.
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 1 g 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 8 phenolic 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 assays for 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 procedure 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} × 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-C18 columns, (A) 50 mm × 4.6 mm.i.d. 3.5 μm, (B) 100 mm × 4.6 mm.i.d. 3.5 μm, (C) 150 mm × 4.6 mm.i.d. 3.5 μm, and (D) 250 mm × 4.6 mm.i.d. 5 μm were tested. The results showed that except for Rg1 and Re, or cryptotanshinone and tanshinone I, the baseline separation for the most compounds studied could be obtained with the four columns by HPLC. Meanwhile, only the base-line separation for 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 Rg1 and Re in 70 min by routine HPLC.
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 R1, (10) ginsenoside Rg1, (11) ginsenoside Re, (12) ginsenoside Rb1, (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 0.56–5.92 μg/ml, 5.92–12.08 μg/ml, 0.59–0.71 μg/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, 1.17–1.41 μg/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 intra-day precision was 0.10–1.11%, especially, it was better to perform the HPLC analysis within 10 h 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 26.86 mg/g except for GP, and the tanshinones ranged from 0.64 to 4.91 mg/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 B in 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 \( y = a + bx \) | 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        |
| Protocatechuclideanhyde | 288           | \( y = 35.142x - 5.5682 \)          | 1.11-300.00          | 0.9988 | 0.55        | 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 | 0.55        | 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 R1 | 203                    | \( y = 1.1736x + 4.6435 \)          | 24.16-1530.00        | 0.9978 | 12.08       | 24.16       |
| Ginsenosides Rg3 | 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 Rb1 | 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       |
| Dihydrorytanshinone 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: peak area of analyte; x: concentration of analyte (μg/mL).
Table 2: Intra-day and inter-day precision of the 17 components

| Analytes                  | Concentration spiked (μg/mL) | Detected (μg/mL) | R.S.D. (%) | Detected (μg/mL) | R.S.D. (%) |
|---------------------------|------------------------------|------------------|------------|------------------|------------|
|                           |                              | Intra-day (n = 4) | Inter-day (n = 3) |
| 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 R₁        | 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 Rg₁          | 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 Rb₁          | 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 * (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 R₁       | 5.20          | 6348.21 | -7.58 | -11.87 | -11.17 | -66.45 | -64.48 | -77.89 |
| Ginsenosides Rg₁         | 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 Rb₁         | 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 |
| Analytes                        | Original mean (µg/g) | Spiked mean (µg/g) | Detected mean (µg/g) | Recovery mean (%) | R.S.D. (%) |
|--------------------------------|----------------------|--------------------|----------------------|-------------------|------------|
| 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 R₁             | 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 Rg₁               | 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 Rb₁               | 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       |
|                                | 2815.39              | 1000.00            | 3754.95              | 93.94             | 3.88       |
|                                | 2864.80              | 900.00             | 3765.02              | 100.02            | 6.63       |
| Analyte                             | GDDP   | FDDP   | FDT    | FDC    | GP     |
|------------------------------------|--------|--------|--------|--------|--------|
| Dannshensu                         | 287.55 | 159.21 | 313.74 | 7948.44| 7504.02|
| Protocatecholic acid               | 26.40  | 26.60  | 21.60  | nd     | 26.90  |
| Protocatechuic aldehyde            | 12.30  | 8.60   | 13.60  | 2987.10| 3011.50|
| Caffeic acid                       | 50.90  | 29.70  | 58.90  | 68.00  | 42.80  |
| Rosmarinic acid                    | 483.80 | 304.70 | 533.70 | 3595.60| 4035.90|
| Lithospermic acid                  | 378.21 | 201.66 | 427.98 | 344.53 | 315.45 |
| Salvinolic acid B                  | 1045.00| 628.50 | 1207.00| 1048.50| 1019.10|
| Salvinolic acid A                  | 125.00 | 78.70  | 138.80 | 2786.90| 2866.60|
| Total                              | 2409.16| 1437.67| 2735.32| 18779.07| 18824.97|
| Notoginsenoside R1                 | 1221.00| 1262.70| 2338.40| 5028.00| 1392.80|
| Ginsenosides Re                    | 6119.50| 4392.20| 4502.30| 1435.40| 2803.70|
| Ginsenosides Rb1                   | 238.06 | 284.07 | 175.25 | nd     | 339.58 |
| Ginsenosides Rd                    | 4040.30| 2971.60| 3922.90| 835.00 | 3257.00|
| Total                              | 26765.66| 26973.07| 26864.65| 10216.60| 10785.60|
| Dihydrotanshinone I                | 56.60  | 25.90  | 55.30  | nd     | 256.70 |
| Cryptotanshinone                   | 665.00 | 606.20 | 741.50 | 4.30   | 1251.70|
| Tanshinone I                       | 489.90 | 321.20 | 515.60 | 20.70  | 1328.30|
| Tanshinone IIA                     | 3212.17| 2230.69| 3598.91| 466.11 | 4034.51|
| Total                              | 4423.67| 3183.99| 4911.31| 491.11 | 555.70 |

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

a Analyte referred to danshensu. b Not detected, Analyte referred to danshensu. 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.

Acknowledgement
This work was supported by the National Nature Science Foundation (No. 81303298 and 81202987) of China and the Fujian Agriculture, Program for New Century Excellent Talents in Fujian Province University (J141128), and Forestry University Foundation for excellent youth teachers (xjq201414).

Financial support and sponsorship
Nil

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Normile D. Asian medicine: The new face of traditional Chinese medicine. Science 2003;299:188-90.
2. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science. Trends Pharmacol Sci 2005;26:558-63.
3. Xue TH, Roy R. Studying traditional Chinese medicine. Science 2003;300:740.
4. Wei YJ, Qi LW, Li P, Luo HW, Yi L, Sheng LH. Improved quality control method for Fufang Danshen preparations through simultaneous determination of phenolic acids, saponins and diterpenoquinones by HPLC coupled with diode array and evaporative light scattering detectors. J Pharm Biomed Anal 2007;45:775-84.
5. Tan J, Guo YY, Zhang HY. Clinical observation on the effect of Guanxin Danshen dripping pills in the treatment of coronary heart disease and angina pectoris. J Emerg Tradit Chin Med 2010;19:1836-7.
6. Huang YC, Xiao ZQ, Chen PJ. Curative effect observation on viral myocarditis treated by Guanxin Danshen dripping pills. MedinoveChina 2010;7:44-5.
7. Cai ZF. Clinical observation on the effect of Guanxin Danshen dripping pills in the treatment of silent myocardial ischemia. Zhong Xi Yi Jie He Xin Nao Xue, Guan Bing, Zazhi, 2007;5:1028-9.
8. Guo C, Yin Y, Duan JL, Zhu YR, Yan JJ, Wei G. et al. Neuroprotective effect and underlying mechanism of sodium danshensu [3-(3,4-dihydroxyphenyl) lactic acid from Radix and Rhizoma Salvi aetmoidis [Danshen]] against cerebral ischemia and reperfusion injury in rats. Phytotherapy 2015;22:283-9.
9. Nabanvi SF, Tenore GC, Daglia M, Tundis R, Loizzo MR, Nabavi SM. The cellular protective effects of rosmarinic acid from bench to bedside. Curr Neurovasc Res 2010;7:44-5.
10. Park JH, Park OK, Yan B, Ahn JH, Kim IH, Lee JC. et al. Neuroprotection via maintenance or increase of antioxidants and neurotrophic factors in ischemic gerbil hippocampus treated with tanshinone I. Chin Med J 2014;127:3396-405.
11. Kim K, Bae ON, Lim KM, Noh JY, Kang S, Chung KY. et al. Novel antiplatelet activity of protocatechuic acid through the inhibition of high shear stress-induced platelet aggregation. J Pharmacol Exp Ther 2012;343:704-11.
12. Park JW, Lee SH, Yang MK, Lee JJ, Song MJ, Ryu SY. et al. 15,16-Dihydrotanshinone I, a major component from salvia miltiorrhizaBunge [Danshan], inhibits rabbit platelet aggregation by suppressing intracellular calcium mobilization. Arch Pharm Res 2008;31:47-53.
13. Maione F, Cantone V, Chini MG, De Feo V, Gasco M, Bifulco G. Molecular mechanism of tanshinone IIA and cryptotanshinone in platelet anti-aggregating effects: an integrated study of pharmacology and computational analysis. Fitoterapia 2015;102:174-8.
14. Moon CY, Ku CR, Cho YH, Lee EJ. Protocatechualdehyde inhibits migration and proliferation of vascular smooth muscle cells and intravascular thrombosis. Biochem Biophys Res Commun 2012;423:116-21.
15. Chang GJ, Chang CJ, Chen WJ, YehYH, Lee HY. Electrophysiological and mechanical effects of caffeic acid phenethyl ester, a novel cardioprotective agent with antiarrhythmic activity, in guinea pig heart. Eur J Pharmacol 2013;702:194-207.
16. Jin CJ, Yu SH, Wang XM, Woo SJ, Park HJ, Lee HC. et al. The effect of lipothemic acid, an antioxidant, on development of diabetic retinopathy in spontaneously obese diabetic rats. PLoS One 2014;9:e98232.
17. Li YJ, Duan CL, Liu JX. Salviaonolic acid A promotes the acceleration of neovascularization in the ischemic rat myocardium and the functions of endothelial progenitor cells. J Ethnopharmacol 2014;151:218-27.
18. Wang M, Sun GB, Sun X, Wang HW, Meng XB, Qin M. et al. Cardioprotective effect of salvinolonic acid B against arsenic trioxide-induced injury in cardiac H9c2 cells via the PI3K/Akt signal pathway. Toxicol Lett 2013;216:100-7.
19. Zhang MQ, Tu JF, Chen H, Shen Y, Pang LX, Yang XH. et al. Janus kinase/signal transducer and activator of transcription inhibitors enhance the protective effect mediated by tanshinone IIA from hypoxic/ischemic injury in cardiac myocytes. Mol Cell Med Rep 2015;11:3115-21.
20. Mao S, Wang L, Zhao X, Shang H, Zhang M, Hinek A. Sodium tanshinone IIA sulfonate for reduction of periprocedural myocardial injury during percutaneous coronary intervention (STAMP trial): Rationale and design. Int J Cardiol 2015;182:329-33.
21. He K, Yan L, Pan CS, Liu YY, Cui YC, Hu BH. et al. ROCK-dependent ATP6 modulation contributes to the protection of notoginsenoside Rg1, against ischemia-reperfusion-induced myocardial injury. Am J Physiol Heart Circ Physiol 2014;307:H1784-76.
22. Wang Y, Li X, Wang X, Lau W, Wang Y, Xing Y. et al. Ginsenoside Rd attenuates myocardial ischemia/reperfusion injury via Akt/GSK-3β signaling and inhibition of the mitochondria-dependent apoptotic pathway. PLoS One 2013;8:e70956.
23. Lim KH, Lim DJ, Kim JH. Ginsenoside-Re ameliorates ischemia and reperfusion injury in the heart: a hemodynamics approach. J Ginseng Res 2013;37:283-92.
24. Wu Y, Xia ZY, Dou J, Zhang L, Xu JJ, Zhao B. et al. Protective effect of ginsenoside Rg3 against myocardial ischemia/reperfusion injury in streptozotocin-induced diabetic rats. Mol Biol Rep 2011;38:4327-35.
25. Xie CL, Li JH, Wang WW, Zheng GQ, Wang LX. Neuroprotective effect of ginsenoside-Rg3 on cerebral ischemia/reperfusion injury in rats by down regulating protease-activated receptor-1 expression. Life Sci 2015;121:145-51.
26. Zhou LM, Chou M, Zuo Z. Improved quality control method for Danshen products-Consideration of both hydrophilic and lipophilic active components. JPharm Biomed Anal 2006;41:744-50.
27. Liu AH, Li L, Xu M, Lin YH, Guo HZ, Guo DA. Simultaneous quantification of six major phenolic acids in the roots of Salvia miltiorrhiza and four related traditional Chinese medicinal preparations by HPLC–DAD method. J Pharm Biomed Anal 2006;41:48-56.
28. Wang ZB, Cao BC, Yu AM, Zhang HQ, Qiu FP. Ultrasound-assisted ionic liquid-based homogeneous liquid-liquid microextraction high-performance liquid chromatography for determination of tanshinones in Salvia miltiorrhiza Bge. Root. J Pharm Biomed Anal 2015;104:97-104.
29. Lu J, Song HP, Li P, Zhou P, Dong X, Chen J. Screening of direct thrombin inhibitors from Radix Salvia miltiorrhiza by a peak fractionation approach. J Pharm Biomed Anal 2015;109:85-90.
30. Cao J, Qi LW, Chen J, Yi L, Li P, Ren MT. et al. Application of liquid chromatography-electrospray ionization time-of-flight mass spectrometry for analysis and quality control of compound Danshen preparations. Biomed Chromatogr 2009;23:397-405.
31. Li WL, Qiu HB. Rapid quantification of phenolic acids in Radix Salviae Miltiorrhizae extract solutions by FTNIR spectroscopy in transflective mode. J Pharm Biomed Anal 2010;52:425-31.
32. Cao J, Li P, Chen J, Tan T, Dai HB. Enhanced separation of Compound Xuexhuangtong capsule using functionalized carbon nanotubes with cationic surfactant solutions in MEEKC. Electrophoresis 2013;34:324-30.
33. Li YG, Song L, Liu M, Hu ZB, Wang ZT, Wang ZT. et al. Saponins from Roots of Panaxnotoginseng. Molecules 2013;18:10352-66.
35. Yuan SM, Ni J, Ke Y. Quantitative determination of salvianolic acid B in Guanxindanshen drop pills by HPLC. Lishizhen Med. Mater. MedRes 2008;19:2439-40.

36. Luo L, Tang DE, Zhu M. RP-HPLC simultaneous determination of notoginsenoside R1, ginsenoside Rg1, and ginsenoside Rb1 in Guanxin Danshen Capsule, Asia-Pac. Tradit Med 2012;8:14-6.

37. Liu HL, Xia L, Cao J, Li P, Qi LW. Simultaneous Determination of twelve saponins in radix et rhizomanotoginseng by rapid resolution LC-ESI-TOF-MS. Chromatographia 2008;68:1033-8.

38. Xia L, Liu HL, Li P, Zhou JL, Qi LW, Yi L et al. Rapid and sensitive analysis of multiple bioactive constituents in Compound Danshen preparations using LC-ESI-TOF-MS. J Sep Sci 2008;31:3156-69.

39. Yao H, Shi PY, Shao Q, Fan XH. Chemical fingerprinting and quantitative analysis of a Panaxnotoginseng preparation using HPLC-UV and HPLC-MS. Chin Med 2011;6:9-.

40. Lai CJS, Tan T, Zeng SL, Dong X, Liu EH, Li P. Relative quantification of multi-components in Panaxnotoginseng (Sanqi) by high-performance liquid chromatography with mass spectrometry using mobile phase compensation. J Pharm Biomed Anal 2015;102:150-6.