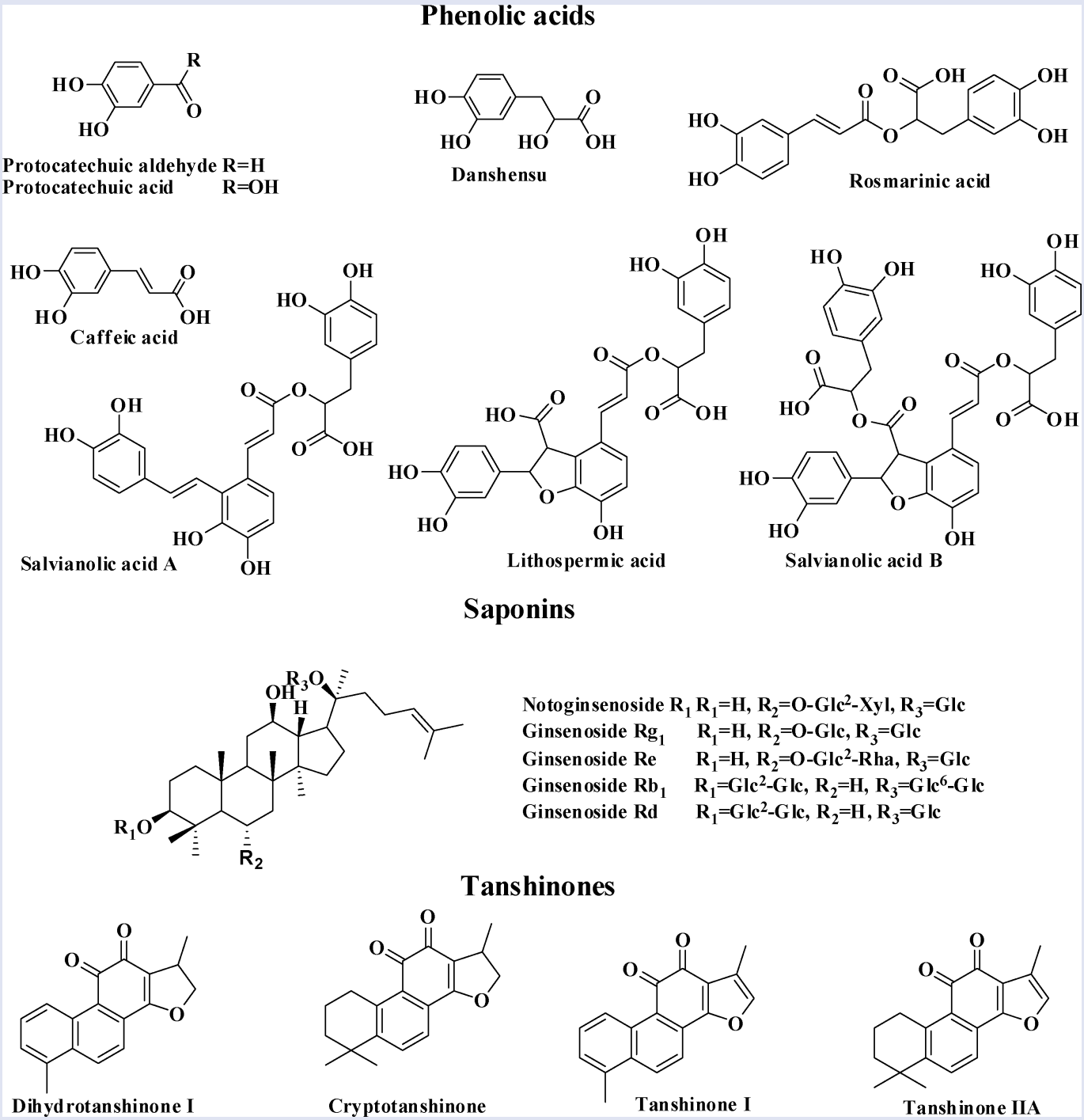

Figure 1: Chemical structures of the 17 compounds.

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.

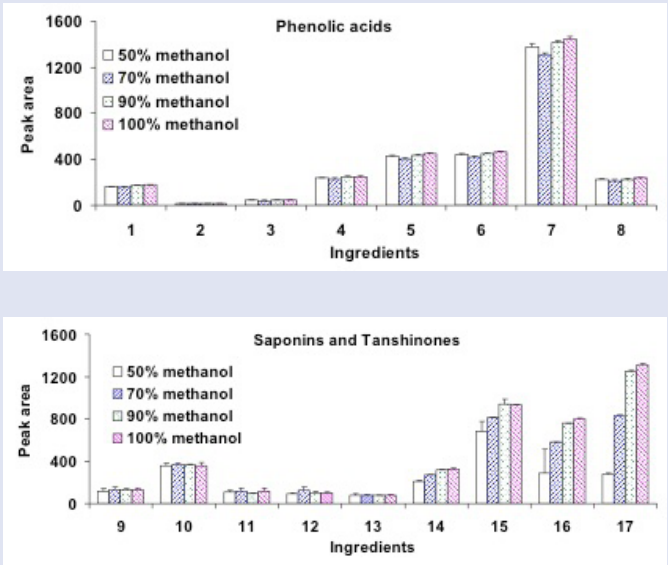

Figure 2: Histograms of peak areas for 17 compounds in GDDP, including danshensu (1), protocatechuic acid (2), protocatechuic aldehyde (3), caffeic acid (4), rosmarinic acid (5), lithospermic acid (6), salvianolic acid B (7), salvianolic acid A (8), notoginsenoside R_1 (9), ginsenosides Rg_1 (10), Re (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).

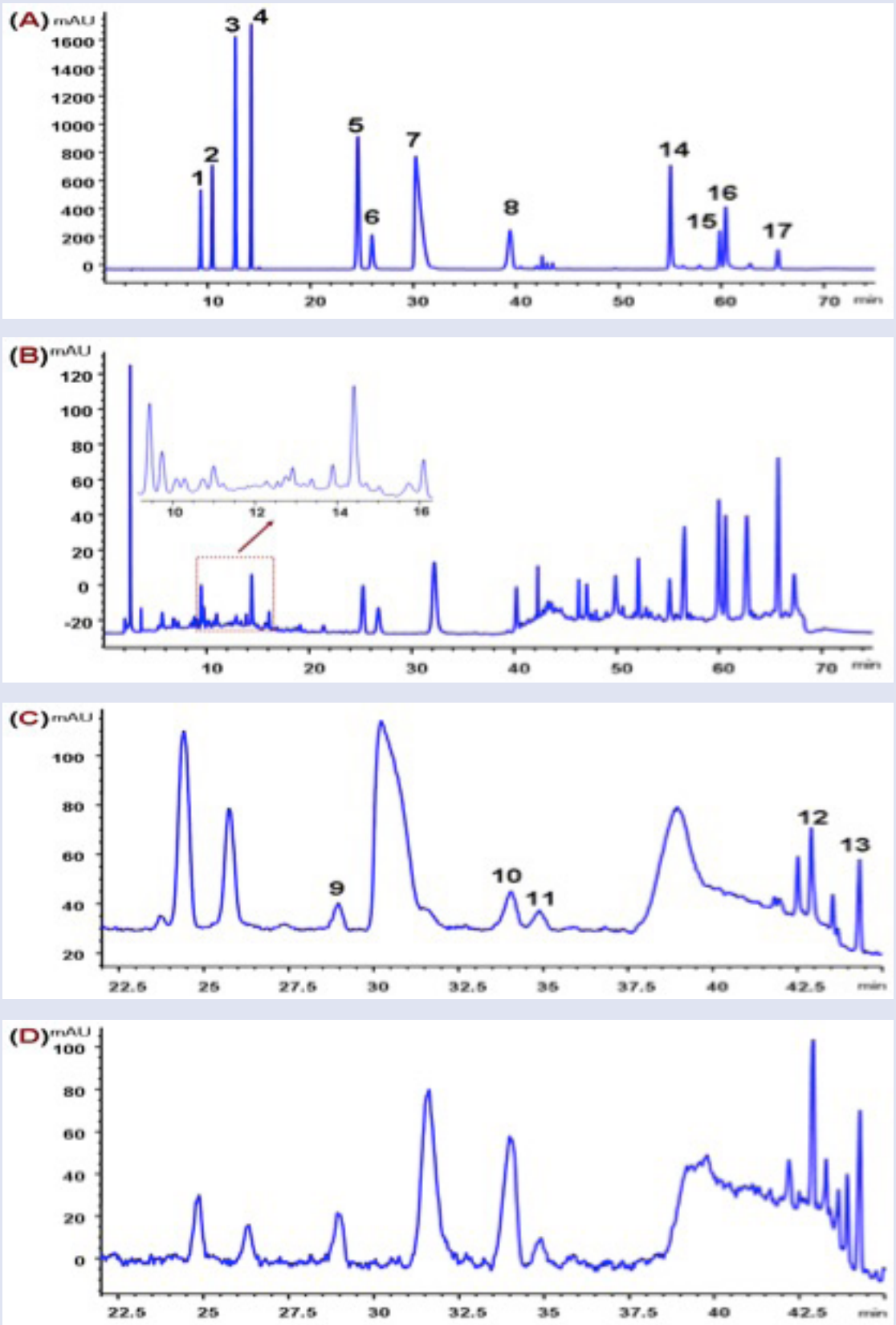

Figure 3: Typical HPLC-DAD chromatograms of 17 standard references (A) and GDDP sample (B) at 288 nm, and standard references (C) and GDDP sample (D) at 203 nm. Peaks (1) sodium danshensu, (2) protocatechuic acid, (3) protocatechuic aldehyde, (4) caffeic acid, (5) rosmarinic acid, (6) lithospermic acid, (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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