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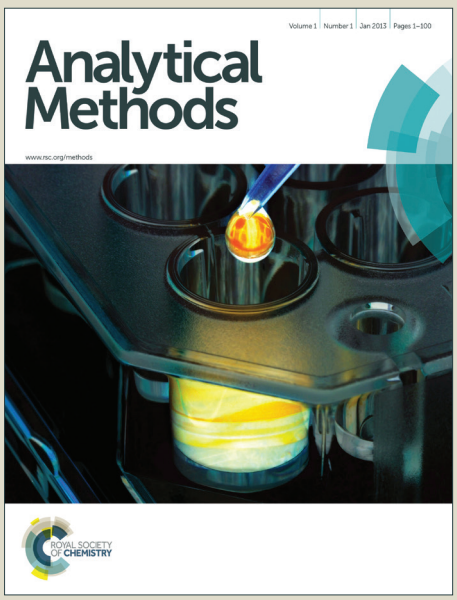

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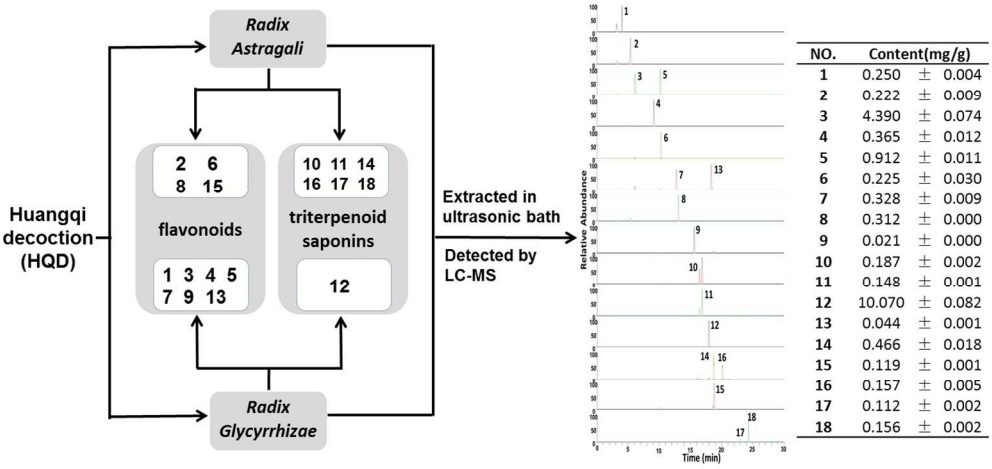

NO.	Content(mg/g)
1	0.250 ± 0.004
2	0.222 ± 0.009
3	4.390 ± 0.074
4	0.365 ± 0.012
5	0.912 ± 0.011
6	0.225 ± 0.030
7	0.328 ± 0.009
8	0.312 ± 0.000
9	0.021 ± 0.000
10	0.187 ± 0.002
11	0.148 ± 0.001
12	10.070 ± 0.082
13	0.044 ± 0.001
14	0.466 ± 0.018
15	0.119 ± 0.001
16	0.157 ± 0.005
17	0.112 ± 0.002
18	0.156 ± 0.002

(1) Schaftoside; (2) Calycosin-7-O-β-D-glucoside; (3) Liquiritin; (4) Isoliquiritin apioside; (5) Isoliquiritin; (6) Ononin; (7) Liquiritigenin; (8) Calycosin; (9) Echinatin; (10) Astragaloside Ⅳ; (11) Astragaloside III; (12) Glycyrrhizic acid; (13) Isoliguiritigenin; (14) Astragaloside Ⅱ; (15) Formononetin; (16) Isoastragaloside Ⅱ; (17) Astragaloside I; (18) Isoastragaloside I.

256x146mm (150 x 150 DPI)

A systematic quality control method of Huangqi decoction: simultaneous determination of eleven flavonoids and seven triterpenoid saponins by ultra high-pressure liquid chromatography coupled with electrospray ionization-mass spectrometry

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ABSTRACT: A novel method of ultra high-pressure liquid chromatography coupled with mass spectrometry (UHPLC-MS) was developed for the quantitative analysis of 18 major bioactive components from Huangqi decoction (HQD). HQD is a classic traditional Chinese medicine (TCM) commonly used to treat consumptive and chronic liver diseases. Chromatographic separation was performed on a reverse-phase C_{18} column for 30 min at a flow rate of 1 mL/min. The optimum mobile phase for the gradient elution was 0.05% aqueous formic acid and acetonitrile. All of the analytes showed good linearity over the tested concentration ranges (r^2 > 0.9972). The recoveries of the three concentration levels ranged from 91.14% to 106.21% with relative standard deviation (RSD) less than 4.69%. Intra- and inter-day precisions were less than 4.73% and 4.97%, respectively. Moreover, this method was successfully used to determine the content of HQD extracts in three different batches. Hence, this method could be used for the multi-component quality control of HQD.

Keywords: Huangqi decoction, bioactive components, UHPLC-MS, quality control

Introduction

Liver fibrosis is a wound-healing response to chronic liver damage caused by liver diseases, which may be due to hepatitis virus, alcohol abuse and nutritional deprivation. Liver fibrosis can further develop into severe hepatopathy, such as hepatocirrhosis and hepatocellular carcinoma. Thus, the development of liver fibrosis should be blocked, inhibited or reversed to treat chronic liver disease. However, the amount of effective medicines for liver fibrosis is insufficient. Chinese herbal medicine has been widely used to treat chronic liver hepatitis and liver cirrhosis for thousands of years. To date, investigations have revealed that traditional Chinese medicine (TCM) exhibits beneficial effects on liver fibrosis [1-3]. Among TCM prescriptions, Huangqi decoction (HQD) is a classical TCM prescribed to treat liver injury since the Song Dynasty (AD 1078) in China. HQD consists of two commonly used medicinal herbs, namely, *Radix Astragali* (RA) and *Radix Glycyrrhizae* (RG), mixed in a ratio of 6/1 (wt/wt). Experimental studies have revealed that HQD elicits a remarkable anti-liver fibrosis effect [4-8]. As such, the bioactive components of HQD should be systematically determined in further research and development. However, no study regarding the component analysis of HQD has been reported.

Triterpenoid saponins and flavonoids are the main bioactive constituents in RA and RG. Hepatoprotective and anti-hepatic fibrosis effects are elicited by triterpenoid saponins, such as astragaloside IV [9-11] and astragaloside extracts containing six constituents (i.e., astragaloside IV, astragaloside III, astragaloside II, isoastragaloside

II, astragaloside I and isoastragaloside I) [12] from RA and glycyrrhizic acid from RG [13, 14], and flavonoids, such as formononetin from RA [15] and liquiritigenin from RG [16-18]. Thus, flavonoids and triterpenoid saponins should be determined from HQD for systematic quality control, safety evaluation, clinical application and investigation of active mechanisms.

Studies have described the methods that can be used to determine the contents of bioactive components in RA or RG simultaneously. Some of these methods can only be used to determine single-class components of one herb; for instance, flavonoids in RA [19] or in RG [20] and saponins in RA [21-23] or RG [24] can be identified. Other methods that may be used to determine flavonoids and triterpenoid saponins simultaneously in RA or RG also have several drawbacks, such as low sensitivity and time consuming using Evaporative Light Scattering Detector (ELSD) [25-27], non-quantitative to astragalosides with weak ultraviolet absorption using DAD detector [28, 29] . Therefore, previously reported methods cannot be applied to determine flavonoids and saponins simultaneously in HQD.

In this study, a novel method of ultra high-pressure liquid chromatography-mass spectrometry (UHPLC-MS) was developed to analyze quantitatively the major bioactive components from HQD (Figure 1). HQD contains eleven flavonoids: schaftoside (1); calycosin-7-O-β-D-glucoside (2); liquiritin (3); isoliquiritin apioside (4); isoliquiritin (5); ononin (6); liquiritigenin (7); calycosin (8); echinatin (9); isoliguiritigenin (13); and formononetin (15). HQD also contains seven saponins: astragaloside IV

(10); astragaloside III (11); glycyrrhizic acid (12); astragaloside II (14); isoastragaloside II (16); astragaloside I (17); and isoastragaloside I (18). The proposed method was successfully applied to determine the amounts of these 18 compounds in three batches of HQD.

2. Experiment

2.1 Materials

HQD extract powder (Batch nos. 1201265, 1212130 and 1212353, 1.2 g equivalent to 6 g of RA crude herbs and 1 g of Radix Glycyrrhizae crude herbs) was prepared by Jiangyin Tianjiang Pharmaceutical Co., Ltd. (China).

The reference standards of astragaloside IV, formononetin and glycyrrhizic acid were purchased from the Chinese National Institute of Control of Pharmaceutical and Biological Products (Beijing, China). Astragaloside I, ononin, calycosin and calycosin-7-O-β-D-glucoside were purchased from Sichuan Weikeqi Biotech Co., Ltd. (Sichuan, China). Astragaloside II and astragaloside III were obtained from Shanghai R&D Center for Standardization of Traditional Chinese Medicines (Shanghai, China). Isoastragaloside I and isoastragaloside II were identified and supplied by Sichuan Xianxin Biotech Co., Ltd. (Sichuan, China). Schaftoside, liquiritin, isoliquiritin apioside, liquiritigenin, isoliguiritigenin and echinatin were purchased from Shanghai Yuanye Bio-Technology Company (Shanghai, China). The purities of these compounds

are >98% according to HPLC analysis results. Acetonitrile and methanol from Burdick&Jackson Company (Ulsan, Korea) and formic acid from Tedia Company (USA) were of HPLC grade. Deionised water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA). The filtration membrane (0.45 μm) were purchased from Millipore Corp. (Bedford, MA, USA). All of the other reagents used were of analytical grade.

2.2 Apparatus and Conditions

Analyses were performed on a Shimadzu UFLC-XR system (Shimadzu, Japan) coupled to an LCQ ion trap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Chromatographic separation was performed on an Agilent Zorbax SB-C18 column (5 μm, 4.6 × 250 mm) at a flow rate of 1 mL/min. The column temperature was maintained at 25 °C. The mobile phase consisted of 0.05% (v/v) formic acid water (A) and acetonitrile (B) with a gradient elution. The process was set as follows: 27% B for 0 min to 3 min; 27% to 66% B for 3 min to 23 min; 66% to 90% B for 23 min to 23.1 min; 90% B for 23.1 min to 25 min; 90% to 27% B for 25 min to 25.1 min; and 27% B for 25.1 min to 30 min.

The mass spectrometer was operated in both positive and negative full-scan modes with a range of mass from 100 *m*/*z* to 1200 *m*/*z*. The detection parameters of the ESI source used were listed as follows: ion spray voltage, 5.0 kV (+) and 4.5 kV (−);sheath gas (N_2) flow rate, 50 arb; capillary voltage, 26 V (+) and −37 V (−); capillary

temperature, 300 °C; auxiliary gas (N_2) flow rate, 13 arb; and tube lens offset, 95 V (+) and −93 V (−).

2.3 Preparation of sample solutions

The HQD powder (25 mg) was extracted with 20 mL of 75% methanol in an ultrasonic bath for 30 min at room temperature (25 °C). After the volume was adjusted to 20 ml, the extracted solution was centrifuged on Scanspeed centrifuge (1730R, LaboGene, Denmark) at 12,000 rpm for 10 min at 4 °C. An aliquot of 20 µL of the supernatant was filtered using a 0.45 µm membrane was injected into the LC system for analysis.

2.4 Preparation of standard solutions

The stock solutions of the 18 reference compounds were accurately weighed and dissolved in methanol. The fresh working solution of the mixture of the 18 reference compounds was prepared by dissolving each of the stock solution in methanol with the following final concentrations of each reference compound: 0.103 (1), 0.151 (2), 2.475 (3), 1.545 (4), 7.438 (5), 0.888 (6), 0.696 (7), 1.200 (8), 0.600 (9), 0.623 (10), 2.920 (11), 1.372 (12), 0.540 (13), 0.766 (14), 0.396 (15), 0.480 (16), 0.668 (17), and 0.500 (18) µg/ml.

2.5 UHPLC-MS method Validation

2.5.1 Calibration Curves, Limits of Detection (LOD) and Limits of Quantification (LOQ)

The working solution, including the 18 reference compounds, was diluted to six suitable concentrations to evaluate the calibration curves. The calibration curves were described by plotting the peak area versus the concentration of each compound. LOD and LOQ were obtained at a signal-to-noise (S/N) ratio of 3 and 10, respectively, by further dilution.

2.5.2 Precision and Accuracy

Intra-day precision within one day and inter-batch precision in three consecutive days were investigated by observing three replicates of each compound at three concentrations (low, middle and high). Accuracy was determined on the basis of the recovered amount of each compound. Three different amounts (low, middle and high) of the 18 reference compounds were added to the HQD sample. The HQD sample was then quantified as described in section 2.2. The recovery of each compound was calculated according to the following equation: recovery (%) = ($amount_{detected}$ – $amount_{original}$) / $amount_{spiked}$ × 100%, where $amount_{detected}$ is the detected total amount of each compound, $amount_{original}$ is the original amount of each compound in HQD and $amount_{spiked}$ is the spiked amount of each compound.

2.5.3 Repeatability and Stability

The repeatability of the method was investigated by detecting 5 extracted solutions

of HQD sample (Batch no. 1201265), and the relative standard deviation (RSD) was used as the standard measure. The stability of the sample was obtained by detecting the same sample solution stored at 4 °C for 0, 6, 12, 24 and 36 h.

3. Results and discussion

3.1 Optimisation of UHPLC-MS conditions

Several UHPLC parameters were optimised for better separation and higher sensitivity in a shorter period. Acetonitrile was chosen as the organic phase because it showed better separation ability than methanol. In addition, different kinds and concentrations of eluent additives were tested, and water containing 0.05% formic acid showed a better peak shape, particularly for glycyrrhizic acid, and a high resolution, particularly for the separation of the most isomeric compounds (i.e., astragaloside III and astragaloside IV). The optimum mobile phase was achieved using acetonitrile with an aqueous phase (containing 0.05% formic acid) in the gradient elution mode.

Different columns, such as Agilent ZorBax Edipse XDB-C18 column (150 × 2.1 mm, 5 µm), Agilent ZorBax SB-C18 column (250 × 4.6 mm, 5 µm) and Thermo ODS-2 HYPERSIL-C18 column (250 × 4.6 mm, 5 µm) were used. Among these used columns, the Agilent ZorBax SB-C18 column (250 × 4.6 mm, 5 µm) showed the best separation. The column temperature was set at 25 °C at a flow rate of 1.0 mL/min to

ensure good separation.

In a full-scan mode, the mass spectral conditions were initially optimised with the reference compounds. In a negative ionisation mode, quasimolequlars ions $[M-H]^-$ of schaftoside, calycosin-7-O-β-D-glucoside, liquiritin, isoliquiritin apioside, isoliquiritin, liquiritigenin, calycosin, echinatin, astragaloside IV, glycyrrhizic acid, isoliguiritigenin, formononetin, isoastragaloside II, astragaloside I and isoastragaloside I were generated. Astragaloside III exhibited adducted molecular ions $[M+CH_3COO]^-$, whereas schaftoside, calycosin-7-O-β-D-glucoside, liquiritin, isoliquiritin apioside, ononin, liquiritigenin, calycosin, echinatin, astragaloside IV, astragaloside III, glycyrrhizic acid, astragaloside II, isoastragaloside II, astragaloside I and isoastragaloside I exhibited protonated molecular ions $[M+H]^+$ in the positive ionisation mode. Some reference compounds exhibited strong signals in both recording modes. Thus, a full-scan mode was applied to determine simultaneously the content of the compounds with electrospray ion source polarity conversion between negative and positive modes in a single run. To achieve the analysis demand, we also optimised several mass spectrum parameters by using the reference compounds based on the lowest interference and the highest signal intensity. The total ion chromatogram of the HQD extracts and the standard mixture solution are shown in Figure 2. The extracted ion chromatograms are shown in Figure 3.

In contrast to apreviously reported method, our proposed method of the simultaneous determination of multiple flavonoids and triterpenoid saponins in HQD

exhibits more sensitivity, shorter time consumption (shortened by threefold)[25-27] and quantitative determination of astragalosides. In addition, our method may prevent the cross-interference of co-existing components, such as isoliquiritin (5) and ononin (6), which were detected in different ion channels, although both compounds displayed the same retention time.

To the best of our knowledge, this study is the first to determine the six astragalosides simultaneously by UHPLC-MS.

3.2 UHPLC-MS method Validation

Each compound in the HQD extracts was identified by comparing the retention time, mass-to-charge ratio and MS^2 with those of each reference standard. All of the compounds were detected in different channels without interfering on another (Figure 3). Figure 4 provides the ms-ms spectra for 18 compounds in the reference standards and Huangqi decoction (HQD) sample. The ms and ms-ms information provides a very solid correlation of standards and the samples. The confirmatory results were sufficient and reliable.

The regression equation for each reference compound, as well as LOD and LOQ values, linear dynamic ranges and mass spectrometry information, are presented in Table 1. All of the compounds showed good linearity (r^2 > 0.9972) in an appropriate concentration range. The LODs and LOQs obtained for flavonoids were 0.2-2.4 ng/mL and 0.5-9.5 ng/mL, respectively, and those of triterpenoid saponins were 1.6-6.3

ng/mL and 6.5-19 ng/mL. According to the previously reported methods that can simultaneously determine the contents of flavonoids and triterpenoid saponins in RA [27] or RG [28] by UV detection or ELSD detection, the LODs and LOQs of flavonoids were 8.58-320 ng/mL and 25.61-600 ng/mL. For the detection of triterpenoid saponins, the LODs and LOQs were 42.90-6200 ng/mL and 123.01-11000 ng/mL. In other words, the proposed MS method in this study is 20 to 550 times more sensitive in terms of LOD and LOQ. Therefore, sensitivity of MS for flavonoids or triterpenoid saponins was higher than that of ELSD or DAD when analyzing flavonoids or triterpenoid saponins and MS showed enough sensitivity for micro-analysis.

The intra- and inter-day precision was less than 4.97% (RSD) (Table 2). The recoveries of the 18 components ranged from 91.1% to 106.2% (RSD < 4.69%; Table 3). The RSD values showing the repeatability of the 18 components were < 4.71%. The samples maintained at 4 °C were stable for 36 h (RSD < 4.42%). These results indicated that the proposed method could be used to determine the 18 biomarkers of HQD simultaneously with high precision, sensitivity and accuracy.

3.3 Extraction Method Development

Two extraction methods, namely, refluxing and ultrasonic bath extraction, were tested to obtain the highest extraction efficiency. The results revealed no significant difference between the two methods; thus, more maneuverable ultrasonic bath extraction was selected. Methanol was chosen as the solvent. Furthermore, different

methanol concentrations (0%, 25%, 50%, 75% and 100%, v/v) were screened, and the triterpenoid saponin yield increased significantly when extractions were performed with 75% methanol. Other factors, such as solvent volume (10, 20 and 30 mL) and extraction times (15, 30, 45 and 60 min), were investigated to optimise the extraction procedure. The results indicated that 25 mg of HQD powder could be extracted completely with 20 mL of 75% methanol in an ultrasonic bath for 30 min only once.

3.4 Sample analysis

The proposed method was applied to analyse 18 compounds in the three batches of HQD samples. Table 4 shows the mean contents of the eleven flavonoids and seven triterpenoid saponins in HQD (n = 3). Although the three batches of HQD samples were from the same pharmaceutical company, the content variation of 15 components was >15%, in which the content variations in four components, such as calycosin-7-O-β-D-glucoside, liquiritigenin, isoliguiritigenin and isoastragaloside I, were >40%. Among the 15 components described in this study, the content variations in seven components, including isoliquiritin apioside, isoliquiritin, glycyrrhizic acid, isoliguiritigenin, isoastragaloside II, astragaloside I and isoastragaloside I may come from the differences between different batches of herbs because the content variations in the two batches (1212130 and 1212353) of the HQD samples prepared from the same batch of RA and RG were <10%. The content variations in the other components, including schaftoside, calycosin-7-O-β-D-glucoside, liquiritin, ononin,

liquiritigenin, calycosin, astragaloside IV and astragaloside II, may come from the preparation process and were 15% to 30% in the two batches (1212130 and 1212353) of the HQD samples. Thus, the content variations in the components of the three batches of HQD samples were mainly due to the different batches of herbs.

The contents of the components in RA [21] and RG [28] may vary with different origins, the contents of Astragaloside I, Astragaloside Ⅱ, Astragaloside Ⅳ, Calycosin-7-O-β-D-glucoside, Calycosin, Ononin, Formononetin were 0.231-1.111 mg/g, 0.128-0.397 mg/g, 0.098-0.430 mg/g, 0.042-0.479 mg/g, 0.006-0.273 mg/g, 0.019-0.126 mg/g and 0.012-0.088 mg/g in eleven commercial *Radix Astragali* samples obtained from various provinces and cities in China, the contents of Liquiritin, Liquiritigenin, Glycyrrhizic acid were 0.13-8.64 mg/g, 0.02-1.30 mg/g and 5.31-29.39 mg/g in 12 Radix Glycyrrhizae samples bought from different cities in China. Therefore, the consistency of the herbal source and the quality control of the preparation process should be considered during the production of HQD samples. In this study, the proposed method provided a basis for a relatively systematic and reliable quality control procedure to ensure the efficacy and safety of HQD products.

4. Conclusions

In this study, a novel, comprehensive and selective method of UHPLC-MS was developed to analyse the major bioactive components of HQD quantitatively for the first time. The method was validated and the results showed that our method is

precise, sensitive and accurate. Using this method, we successfully quantified 18 compounds in HQD and provided a reliable procedure for HQD quality control.

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Table 1. Calibration curves, LOD and LOQ of the 18 analytes

No	Calibration curve[a]	r^2	Linear range (μg/mL)	LOQ[b] (ng/mL)	LOD[c] (ng/mL)	M/Z	Detected ion
1	Y = 71.2063+44907*X	0.9992	0.003 - 0.103	0.5	0.2	563.40	$[M-H]^-$
2	Y = 480.03+122704*X	0.9990	0.004 - 0.151	1.0	0.3	446.90	$[M+H]^+$
3	Y = 354.709+21922.9*X	0.9991	0.062 - 2.475	9.5	2.4	417.30	$[M-H]^-$
4	Y = 185.689+19048*X	0.9976	0.039 - 1.545	5.2	1.7	549.50	$[M-H]^-$
5	Y = -280.725+7056.78*X	0.9976	0.372 - 7.438	9.3	2.3	417.30	$[M-H]^-$
6	Y = 361.671+57896*X	0.9972	0.022 - 0.888	3.1	1.1	475.05	$[M-H]^-$
7	Y = 94.2926+18480.3*X	0.9995	0.017 - 0.696	5.5	1.8	255.20	$[M+H]^+$
8	Y = 1098.24+81218.4*X	0.9980	0.030 - 1.200	2.4	0.8	283.10	$[M-H]^-$
9	Y = 626.516+58249.6*X	0.9990	0.015 - 0.600	1.4	0.4	269.20	$[M-H]^-$
10	Y = 76.4558+281228*X	0.9992	0.016 - 0.623	8.0	2.7	784.50	$[M+H]^+$
11	Y = -2408.36+109810*X	0.9983	0.073 - 2.920	10.0	3.3	829.50	$[M+HCOO]^-$
12	Y = -1897.95+179517*X	0.9982	0.034 - 1.372	1.7	0.6	821.80	$[M-H]^-$
13	Y = 241.539+81914.7*X	0.9982	0.014 - 0.540	2.3	0.8	255.20	$[M-H]^-$
14	Y = -255.704+140686*X	0.9987	0.019 - 0.766	19.0	6.3	826.70	$[M+H]^+$
15	Y = 762.746+119426*X	0.9985	0.010 - 0.396	1.3	0.4	267.25	$[M-H]^-$
16	Y = 98.3882+204025*X	0.9990	0.012 - 0.480	10.0	3.3	826.70	$[M+H]^+$
17	Y = -1207.91+215141*X	0.9986	0.017 - 0.668	17.0	5.7	868.55	$[M+H]^+$
18	Y = -1271.55+280920*X	0.9989	0.013 - 0.500	6.5	1.6	868.55	$[M+H]^+$

a. Y is the peak area of mass detection, X is the compound concentration injected and r^2 is the correlation determination of the equation.

b. LOD refers to the limits of detection

c. LOQ refers to the limits of quantification

Table 2. Intra- and inter-day variability and repeatability for the assay of the 18 analytes

NO.	Concentration (μg/ml)	Intra-day (n = 3) Found	Intra-day (n = 3) RSD (%)	Inter-day (n =3) Found	Inter-day (n =3) RSD (%)	Repeatability (n = 5) Found	Repeatability (n = 5) RSD (%)
1	0.010	0.010 ± 0.000	4.20	0.011 ± 0.000	1.97	0.061 ± 0.003	4.71
	0.041	0.042 ± 0.001	1.36	0.042 ± 0.001	1.59		
	0.103	0.102 ± 0.003	2.97	0.102 ± 0.005	4.97		
2	0.015	0.151 ± 0.000	1.84	0.015 ± 0.001	3.32	0.139 ± 0.007	4.68
	0.060	0.062 ± 0.001	1.14	0.063 ± 0.001	0.94		
	0.151	0.149 ± 0.003	2.17	0.150 ± 0.001	0.92		
3	0.248	0.244 ± 0.009	3.57	0.252 ± 0.005	2.11	0.251 ± 0.010	4.15
	0.990	1.008 ± 0.014	1.34	1.012 ± 0.012	1.20		
	2.475	2.409 ± 0.051	2.13	2.407 ± 0.049	2.05		
4	0.155	0.159 ± 0.005	3.25	0.159 ± 0.000	0.14	0.347 ± 0.010	2.84
	0.618	0.641 ± 0.027	4.19	0.629 ± 0.002	0.37		
	1.545	1.486 ± 0.048	3.24	1.483 ± 0.019	1.26		
5	0.744	0.758 ± 0.042	4.37	0.755 ± 0.044	4.13	1.291 ± 0.029	2.24
	2.975	2.998 ± 0.093	3.09	2.982 ± 0.094	3.12		
	7.438	7.213 ± 0.010	0.14	7.088 ± 0.127	1.79		
6	0.089	0.094 ± 0.001	0.53	0.090 ± 0.001	1.61	0.260 ± 0.004	1.49
	0.355	0.359 ± 0.006	1.61	0.363 ± 0.003	0.77		
	0.888	0.840 ± 0.024	2.87	0.850 ± 0.017	1.96		
7	0.070	0.070 ± 0.001	1.13	0.072 ± 0.001	1.07	0.050 ± 0.002	4.41
	0.278	0.283 ± 0.003	0.88	0.287 ± 0.009	3.27		
	0.696	0.683 ± 0.014	2.09	0.666 ± 0.017	2.61		
8	0.120	0.125 ± 0.001	1.10	0.124 ± 0.005	3.84	0.255 ± 0.002	0.71
	0.480	0.494 ± 0.000	0.03	0.494 ± 0.006	1.13		
	1.200	1.142 ± 0.017	1.48	1.134 ± 0.028	2.49		
9	0.060	0.060 ± 0.001	1.75	0.061 ± 0.001	1.83	0.031 ± 0.001	1.72
	0.240	0.245 ± 0.001	0.29	0.245 ± 0.003	1.08		
	0.600	0.592 ± 0.006	1.07	0.581 ± 0.006	1.00		
10	0.062	0.061 ± 0.001	2.08	0.063 ± 0.001	1.67	0.274 ± 0.006	2.06
	0.249	0.253 ± 0.005	2.03	0.253 ± 0.005	2.12		
	0.623	0.626 ± 0.020	3.18	0.610 ± 0.023	3.77		
11	0.292	0.269 ± 0.000	0.16	0.276 ± 0.011	3.95	0.249 ± 0.009	0.98
	1.168	1.235 ± 0.015	1.23	1.215 ± 0.020	1.63		
	2.920	2.859 ± 0.027	0.93	2.880 ± 0.022	0.77		
12	0.137	0.140 ± 0.002	1.56	0.136 ± 0.003	2.35	0.452 ± 0.007	1.51
	0.549	0.570 ± 0.002	0.38	0.562 ± 0.016	2.84		
	1.372	1.328 ± 0.023	1.71	1.345 ± 0.040	2.99		

13	0.054	0.056	±	0.001	1.21	0.056	±	0.003	4.47	0.187	± 0.002	0.94
	0.216	0.220	±	0.002	0.81	0.217	±	0.007	3.05			
	0.540	0.520	±	0.009	1.77	0.507	±	0.242	4.77			
14	0.077	0.079	±	0.001	1.67	0.077	±	0.001	1.09	0.718	± 0.018	2.44
	0.306	0.310	±	0.005	1.54	0.299	±	0.014	4.70			
	0.766	0.751	±	0.016	2.07	0.791	±	0.037	4.64			
15	0.040	0.042	±	0.001	2.41	0.397	±	0.001	1.99	0.140	± 0.002	1.24
	0.158	0.163	±	0.001	0.84	0.163	±	0.002	1.03			
	0.396	0.393	±	0.015	3.70	0.387	±	0.012	2.97			
16	0.048	0.047	±	0.000	0.68	0.047	±	0.001	2.01	0.283	± 0.006	2.12
	0.192	0.200	±	0.003	1.42	0.191	±	0.006	3.33			
	0.480	0.476	±	0.011	2.33	0.484	±	0.013	2.62			
17	0.067	0.067	±	0.001	1.03	0.066	±	0.002	3.51	0.268	± 0.005	1.97
	0.267	0.263	±	0.012	4.40	0.268	±	0.008	3.06			
	0.668	0.667	±	0.032	4.73	0.688	±	0.009	1.35			
18	0.050	0.049	±	0.001	2.40	0.048	±	0.002	4.34	0.492	± 0.016	3.27
	0.200	0.195	±	0.003	1.51	0.199	±	0.005	2.71			
	0.500	0.507	±	0.022	4.24	0.507	±	0.015	2.98			

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Table 3. Recovery of the 18 analytes in Huangqi decoction (n = 3)

No.	Spiked amount (μg)	Found (μg)	Recovery (%)	RSD (%)
1	0.051	0.052 ± 0.001	102.45	1.720
	0.084	0.081 ± 0.001	95.57	1.007
	0.118	0.120 ± 0.002	101.55	1.952
2	0.154	0.158 ± 0.002	102.18	1.161
	0.256	0.249 ± 0.005	97.22	2.109
	0.359	0.327 ± 0.007	91.14	2.114
3	0.124	0.120 ± 0.008	99.93	1.720
	0.206	0.201 ± 0.004	97.75	1.007
	0.288	0.274 ± 0.006	91.53	1.952
4	0.296	0.295 ± 0.002	99.91	0.777
	0.492	0.495 ± 0.007	100.65	1.314
	0.690	0.708 ± 0.014	102.78	1.900
5	0.774	0.831 0.025	104.93	3.279
	1.291	1.337 0.027	103.55	2.098
	1.807	1.835 0.022	100.81	1.885
6	0.133	0.134 ± 0.002	100.63	1.110
	0.222	0.217 ± 0.004	97.70	2.036
	0.310	0.291 ± 0.008	93.47	2.695
7	0.270	0.268 ± 0.011	99.27	4.099
	0.450	0.459 ± 0.008	102.10	1.648
	0.630	0.631 ± 0.001	100.18	0.268
8	0.281	0.285 ± 0.006	101.71	2.089
	0.467	0.466 ± 0.018	99.80	3.780
	0.653	0.627 ± 0.011	95.82	1.623
9	0.019	0.018 ± 0.001	99.20	2.373
	0.031	0.031 ± 0.001	100.38	1.927
	0.043	0.043 ± 0.001	100.56	2.703
10	0.179	0.180 ± 0.005	100.53	2.960
	0.298	0.306 ± 0.004	102.79	1.146
	0.418	0.424 ± 0.006	101.51	1.377
11	0.117	0.117 ± 0.003	100.17	2.564
	0.195	0.201 ± 0.002	103.42	1.147
	0.273	0.279 ± 0.009	102.17	3.489
12	0.311	0.306 ± 0.029	104.14	0.328
	0.517	0.523 ± 0.012	101.09	2.202
	0.725	0.726 ± 0.019	100.19	2.632
13	0.044	0.045 ± 0.002	101.24	4.218
	0.074	0.071 ± 0.003	96.83	3.528

	0.103	0.098	±	0.002	95.02	2.381
14	0.306	0.302	±	0.009	98.68	2.870
	0.510	0.509	±	0.013	99.80	2.618
	0.714	0.690	±	0.006	96.63	0.879
15	0.077	0.077	±	0.002	100.46	2.459
	0.127	0.126	±	0.001	99.34	1.042
	0.179	0.177	±	0.005	99.68	2.314
16	0.121	0.117	±	0.019	106.21	0.415
	0.201	0.191	±	0.003	95.35	1.680
	0.281	0.261	±	0.004	93.16	1.122
17	0.095	0.095	±	0.002	100.05	2.362
	0.159	0.160	±	0.009	101.68	4.693
	0.222	0.222	±	0.004	99.79	1.393
18	0.145	0.143	±	0.002	99.07	1.047
	0.241	0.246	±	0.005	101.94	2.089
	0.337	0.354	±	0.002	104.77	0.660

375 **Table 4**. Contents of the 18 analytes in Huangqi decoction

NO.	Content (mg/g)		
	Batch no.1201265	Batch no.1212130	Batch no.1212353
1	0.250 ± 0.004	0.239 ± 0.016	0.182 ± 0.013
2	0.222 ± 0.009	0.567 ± 0.034	0.392 ± 0.030
3	4.390 ± 0.074	3.683 ± 0.184	2.399 ± 0.081
4	0.365 ± 0.012	0.261 ± 0.008	0.250 ± 0.001
5	0.912 ± 0.011	0.664 ± 0.011	0.632 ± 0.014
6	0.225 ± 0.030	0.190 ± 0.006	0.152 ± 0.006
7	0.328 ± 0.009	0.814 ± 0.014	0.571 ± 0.002
8	0.312 ± 0.000	0.197 ± 0.001	0.269 ± 0.001
9	0.021 ± 0.000	0.025 ± 0.000	0.025 ± 0.000
10	0.187 ± 0.002	0.228 ± 0.001	0.335 ± 0.008
11	0.148 ± 0.001	0.200 ± 0.004	0.179 ± 0.006
12	10.070 ± 0.082	7.278 ± 0.150	6.629 ± 0.133
13	0.044 ± 0.001	0.153 ± 0.002	0.146 ± 0.006
14	0.466 ± 0.018	0.560 ± 0.011	0.848 ± 0.021
15	0.119 ± 0.001	0.112 ± 0.003	0.124 ± 0.003
16	0.157 ± 0.005	0.227 ± 0.007	0.197 ± 0.005
17	0.112 ± 0.002	0.205 ± 0.006	0.209 ± 0.016
18	0.156 ± 0.002	0.435 ± 0.022	0.448 ± 0.024

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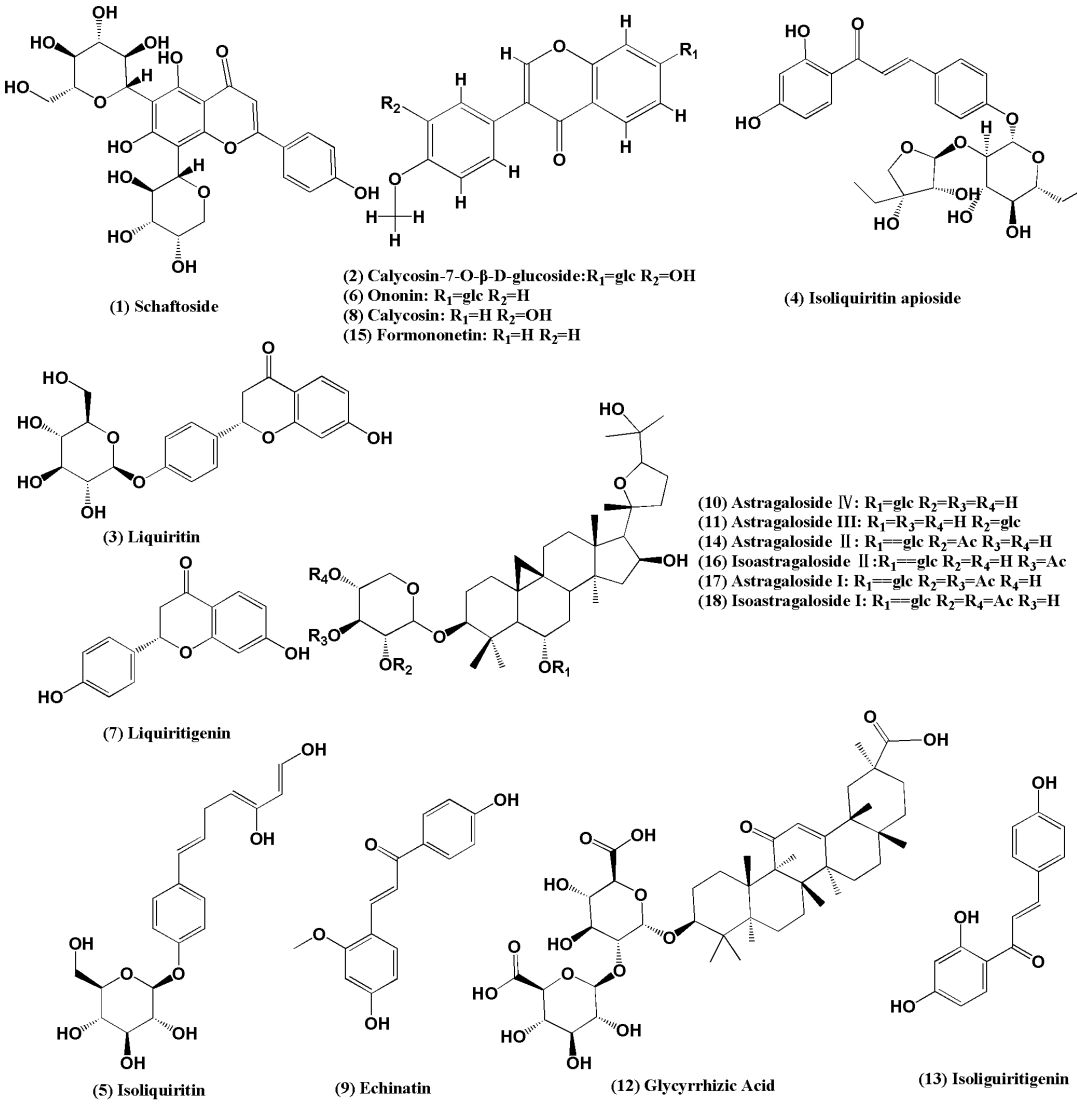

Figure 1. Chemical structures of the 18 analytes in Huangqi decoction.

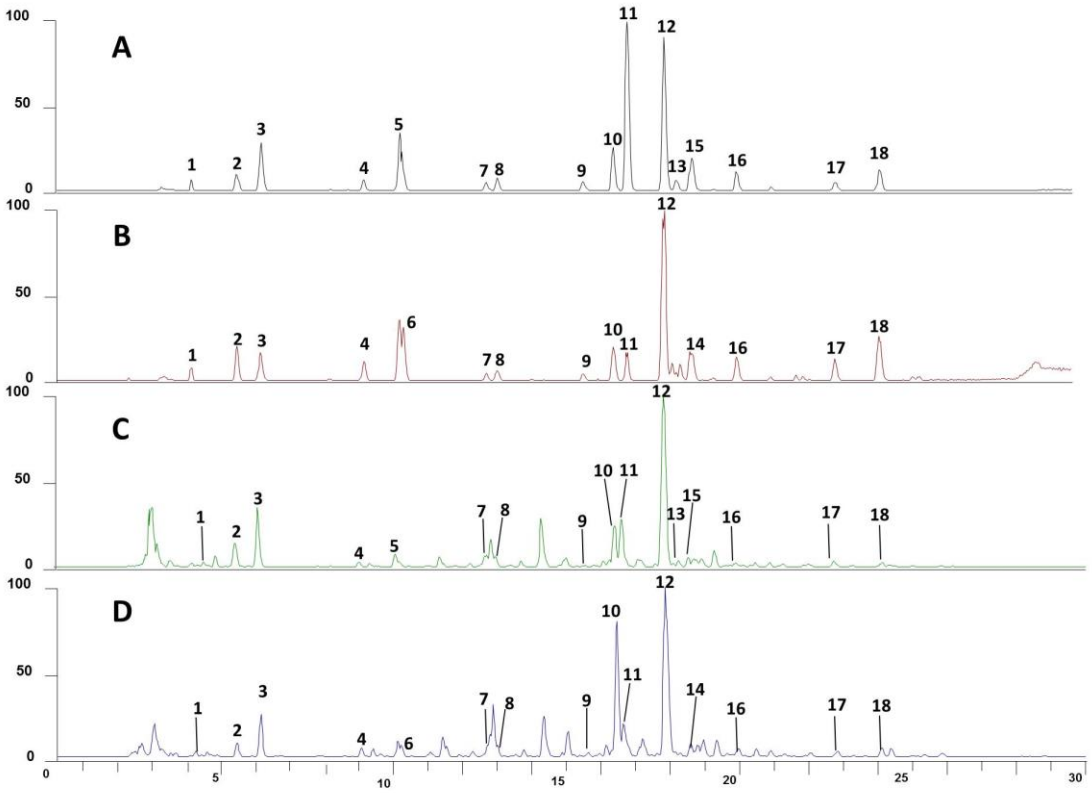

Figure 2. Total ion chromatograms (TIC) of the reference standards and Huangqi decoction (HQD) samples. (A) TIC of the reference standards in negative ion mode; (B) TIC of the reference standards in positive ion mode; (C) TIC of the HQD sample in negative ion mode; (D) TIC of HQD sample in positive ion mode: (1) Schaftoside; (2) Calycosin-7-O-β-D-glucoside; (3) Liquiritin; (4) Isoliquiritin apioside; (5) Isoliquiritin; (6) Ononin; (7) Liquiritigenin; (8) Calycosin; (9) Echinatin; (10) Astragaloside IV; (11) Astragaloside III; (12) Glycyrrhizic acid; (13) Isoliguiritigenin; (14) Astragaloside II; (15) Formononetin; (16) Isoastragaloside II; (17) Astragaloside I; (18) Isoastragaloside I.

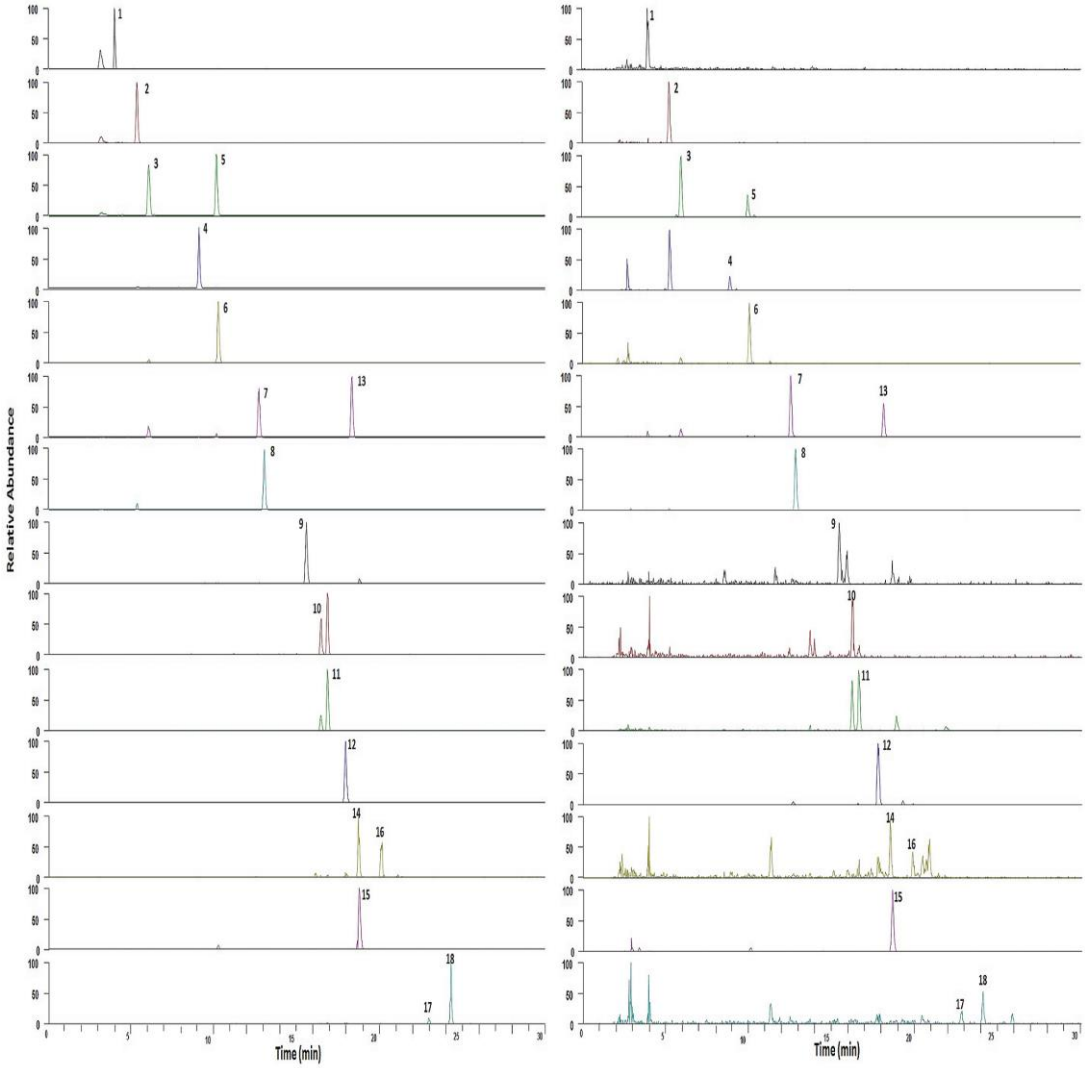

Figure 3. Extracted ion chromatograms of the reference standards (A) and Huangqi decoction (HQD) samples (B): (1) Schaftoside; (2) Calycosin-7-O-β-D-glucoside; (3) Liquiritin; (4) Isoliquiritin apioside; (5) Isoliquiritin; (6) Ononin; (7) Liquiritigenin; (8) Calycosin; (9) Echinatin; (10) Astragaloside IV; (11) Astragaloside III; (12) Astragaloside II; (13) Isoliguiritigenin; (14) Astragaloside II; (15) Formononetin; (16) Isoastragaloside III; (17) Astragaloside I; (18) Isoastragaloside I.

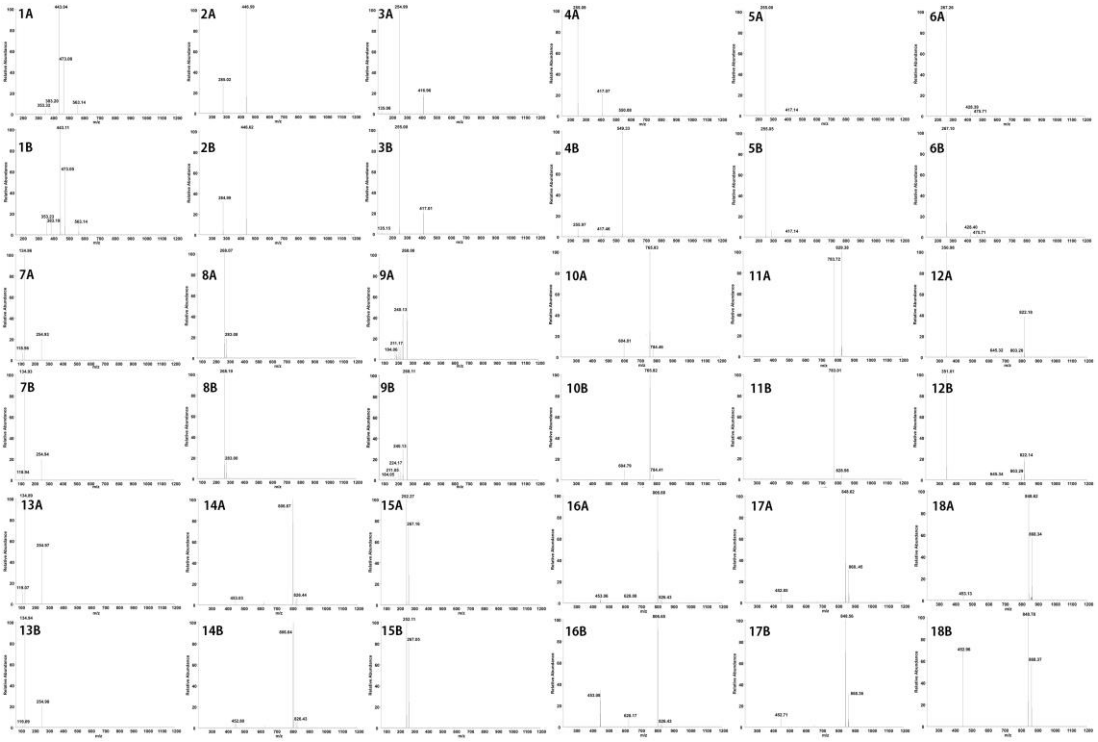

Figure 4. the MS^2 spectra of the reference standards (A) and Huangqi decoction (HQD) samples (B): (1) Schaftoside; (2) Calycosin-7-O-β-D-glucoside; (3) Liquiritin; (4) Isoliquiritin apioside; (5) Isoliquiritin; (6) Ononin; (7) Liquiritigenin; (8) Calycosin; (9) Echinatin; (10) Astragaloside IV; (11) Astragaloside III; (12) Astragaloside II; (13) Isoliguiritigenin; (14) Astragaloside II; (15) Formononetin; (16) Isoastragaloside III; (17) Astragaloside I; (18) Isoastragaloside I.