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Concurrent identification of 11 major primary active compounds in Huangqin Qingfei Decoction by liquid chromatography tandem mass spectrometry via liquid chromatography tandem mass spectrometry

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Abstract: Huangqin Qinfei Decoction (HQD) is a traditional Chinese medicine that is administered for acute pneumonia, bronchial inflammation, acute bronchitis and acute lung infection. In this study, we used liquid chromatography linked with tandem mass spectrometry (LC-MS/MS) for the concurrent identification of 11 bioactive compounds; namely, baicalin, baicalein, wogonoside, scutellarin, wogonin, oroxylin A, geniposide, genipin, geniposidic acid, chlorogenic acid, and crocin-I, for the quality control of HQD. The evaluation was conducted on an Agilent Poroshell 120 EC-C18 (2.1mm×100mm, 2.7μm) with gradient elution in the mobile phase with 0.1% formic acid and 1mM/L ammonium acetate in water as solvent A and methanol as solvent B at a flow rate of 0.3mL/min in under 12 min. Mass spectrometric detection was conducted in the selected reaction monitoring mode utilizing electro spray ionization in the positive and negative modes. Every one of the calibration curves had good linearity with R^2 >0.9992. Intra-day and inter-day accuracies for every one of the evaluated components were expressed as the relative standard deviation (RSD) from 1.72%-5.02% and 0.63%-5.99%, respectively. The recuperation of the 11 compounds that were measured at the three concentrations was within 94.05%-105.18%, with the RSD ≤ 6.26%. The use of this method was determined through the effective evaluation of 11 compounds in 5 batches of HQD. The confirmed method is precise, sensitive, and effective for identifying the contents of the chosen compounds in HQD for quality control.

Keywords: Huangqin Qinfei Decoction, LC-MS/MS, simultaneous determination, quality control.

INTRODUCTION

Huangqin Qingfei Decoction (HQD) is a classical prescription in traditional Chinese medicine from the Precious Mirror of Hygiene since the Yuan Dynasty (AD 1206-1368) for the treatment of clearing heat and removing toxicity. It has been widely used to treat acute pneumonia, acute bronchitis and bronchial inflammation in the modern clinic (Cheng GQ, 2016, Wang, Li *et al*., 2013, Yao, He *et al.,* 2006). HQD is comprised of two herbal medicines, *Radix Scutellaria* and *Fructus gardeniae*. Flavonoids, iridoids, caffeoyl quinic acid derivatives, and crocins are considered to be the main pharmacological components of HQD (Li, Lin *et al.,* 2011, Yu, Xie *et al.,* 2009). As reported previously, flavonoids, such as baicalin, baicalein, wogonoside, scutellarin, wogonin, and oroxylin A, are the major ingredients in *Radix Scutellariae* and they have been proven to possess a variety of pharmacological effects, including anti-inflammation (Huang, Lee *et al.,* 2006), anti-cancer (Li 2009, Sun, Lu, Ling *et al.,* 2009), anti-hepatitis (Guo, Zhao *et al.,* 2007, Gao, Zhao *et al.,* 2016), neuroprotective (Wang, Ma *et al.,* 2016), anti-viral, and anti-influenza effects. (Nayak, Agrawal *et al.,* 2014) Meanwhile, iridoid glycosides (geniposide, genipin, and geniposidic acid), caffeoyl quinic acid derivatives (chlorogenic acid), and croins (crocin-I), which contribute to the pharmacological efficacy, are present in *Fructus gardeniae* and they exert plentiful biological effects, such as anti-inflammatory (Koo, Lim *et al.,* 2006, Song, Zhang *et al.,* 2014), anti-diabetic (Yao, Shu *et al.,* 2014), antithrombotic (Zhang, Liu *et al.,* 2017), anti-tumor (Hsu, Yang *et al.,* 1997), anti-allergic (Sung, Lee *et al.,* 2014), and antioxidant effects (Chen, Zhang *et al.,* 2008). Up to now, quantitative analyses of major constituents in HQD and its two herbs, *Radix Scutellaria* and *Fructus gardenia*, have been carried out using high performance liquid chromatography (HPLC) or Ultra high performance liquid chromatography (UHPLC) (Zhang, Zhao *et al.,* 2008, Li, Zhou, *et al.,* 2009, Cui, Cai *et al.,* 2016, Ouyang, Zhang *et al.,* 2011, Lee, Hong *et al.,* 2014), high performance capillary electrophoresis (HPCE) (Han, Cheng *et al.,* 2000), near-infrared spectrum (NIR) (Navarro, Rodenas *et al.,* 2013), and liquid chromatography-mass spectrometry (HPLC-MS) (Wu, Zhou *et al.,* 2013). However, these methods suffer from a long run time, they are not accurate enough or only measure the flavones and the iridoids in *Radix Scutellaria* and *Fructus gardeniae* separately; the simultaneously quantitative determination of 11 active compounds, namely, baicalin, baicalein, wogonoside, scutellarin, wogonin, oroxylin A, geniposide, genipin, geniposidic acid, chlorogenic acid and crocin-I as shown in fig. 1 of HQD has not been reported. Thus, the objective of our research was to create an effective, sensitive, and selective method utilizing liquid chromatography linked with tandem mass spectrometry

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(LC-MS/MS) through the optimization of the extraction, separation, and analytical settings for the concurrent identification of 11 primary parts of HQD, and the method can be used for the quality control of HQD.

MATERIALS AND METHODS

Reagents and materials

Standard baicalin, baicalein, wogonin, scutellarin, geniposide, chlorogenic acid, and carbamazepine (internal standard) were acquired from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Wogonoside and oroxylin A were purchased from Shanghai Ronghe Medical Technology Development Co. Ltd. (Shanghai, China). Genipin, geniposidic acid, and crocin-I were bought from Shanghai Dingrui Chemicals Co. Ltd. (Shanghai, China). The purities of every one of the reference standards were higher than 98.0% based on HPLC evaluation. Chromatographic grade formic acid, methanol, and acetonitrile came from Merck Company (Darmstadt, Germany), and acetic acid (HPLC grade; purity, ≥ 99.7%) was bought from Tedia Company (USA), and they were utilized in the sample preparation. Deionised water was cleansed with a Milli-Q system (Millipore, Bedford, MA, USA). Crude *Radix Scutellariae* (Hebei, China) and *Fructus Gardeniaes Ellis* (Jiangxi, China) were bought from Kangqiao Medicinal Materials Electuary Co., Ltd. (Shanghai, China).

Instrumentation and chromatographic system

Analytes were examined on a Shimadzu Prominence UFLCXR system (Shimadzu Scientific Instruments, Kyoto, Japan) linked to a Thermo Scientific TQS Quantum Ultra triple-quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with an electrospray ionization source functioning in the chosen reaction monitoring mode detection.

The electrospray capillary voltage was put at 3000V (+) or 2500V (-), and the vaporizer and capillary temperature were kept at 300°C. The sheath and auxiliary gas pressures were 40 and 10 psi, respectively. The auxiliary gas flow rate was 8L/min, and argon was utilized as the collision gas, with the collision pressure put at 1.5m Torr. Collision energy (CE) and the precursor-to-product ion pairs for every part are included in table 1.

Chromatographic separation was conducted on an Agilent Poroshell120 EC-C18 analytical column (2.1×100mm, 2.7μm), and it was kept at 30°C. We used a gradient elution system of 0.1% formic acid and 1mmol/L ammonium acetate in water as solvent A and methanol as solvent B at a flow rate of 0.3mL/min in a run time of 12.0min. The gradient steps were: 0-2min, 30% B→40% B; 2-3.5min, 40% B→90% B; 3.5-6.0min, 90% B→90% B; 6.0-7.0min, 90% B→60% B; 7.0-10.0min, 60% B→40% B; and 10.0min- 12min, 40% B. The injection volume was 10μL.

Standard solutions and sample preparation

A solution of the 11 standard reference compounds and the internal standard (IS) was prepared by dissolving precisely weighed parts of the standards in methanol, and then a standard mixture solution was acquired by accurately combining the 11 stock solutions with methanol so that the concentrations of baicalin, baicalein, wogonoside, scutellarin, wogonin, oroxylin A, geniposide, genipin, geniposidic acid, and chlorogenic acid crocin-I1 were 408.0, 660.0, 450.0, 354.0, 69.0, 33.0, 1020.0, 57.0, 84.0, 138.0, and 78.0μg/mL, respectively. The IS solution of carbamazepine was also diluted with methanol to generate a dernier concentration of 500ng/mL. This was then diluted to set up 6 solutions with various concentrations to create calibration curves. Every one of the solutions was kept in a refrigerator at 4°C for evaluation. Prior to the assessment, they were filtered through a 0.22-μm membrane filter.

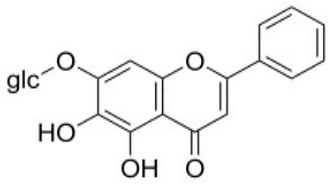
Crude *Radix Scutellaria* (20g) and *Fructus Gardeniae* (6g) were submerged in a 10-fold mass of water for 30 min, and decocted for 1h. Following filtration, the residue was then added with an 8-fold mass of water and boiled for another 1h, filtrated, combined with the two decoctions, and then freeze-dried to acquire the powdered HQD. Then, an aliquot of 1.0g of powder, which had been precisely weighed, was removed over 30 min with 100mL of 80% methanol in an ultrasonic water bath. Following that, the extract of 1.00mL filtrate above and the IS solution of 1.00mL was accurately drawn into a brown volumetric flask of 10mL and 80% methanol was also added to the volume. Finally, the prepared sample solution was put through a 0.22-μm membrane prior to injection. Then, 10μL of each filtrated solution was injected into the LC system for evaluation. The test decoctions HQD1 (batch no. ZY21301), HQD2 (batch no. ZY21302), HQD3 (batch no. ZY21303), HQD4 (batch no. ZY21304), and HQD5 (batch no. ZY21305) were prepared in our laboratory from various batches of the two crude drugs.

STATISTICAL ANALYSIS

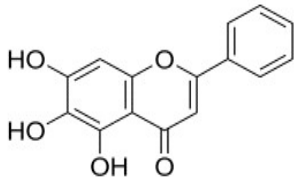
All data analysis was performed using Xcalibur software version 4.1 (Thermo Electron, Waltham, USA).

Method validation

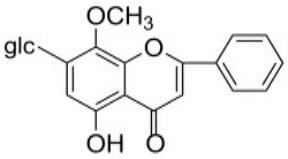
The linearity of each calibration curve was established by plotting the peak area ratio (y) of the analytes to IS *vs* the nominal concentration (x) of the analytes with weighted ($1/x^2$) least square linear regression. The sensitivity was examined by the limit of detection (LOD) and limit of quantification (LOQ), providing a signal-to-noise (S/N) ratio of 3 and 10, respectively. Standard solutions with 7 various concentration levels were set by additional dilution of the stock solution with 80% methanol to determine linearity, LOD and LOQ.



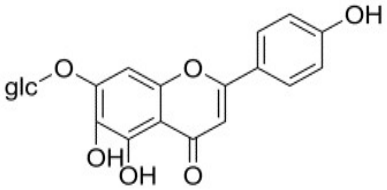
Baicalin; mol.wt. 446



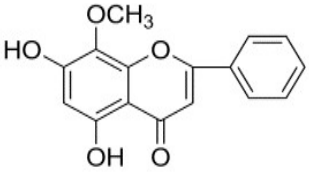
Baicalein; mol. wt.270



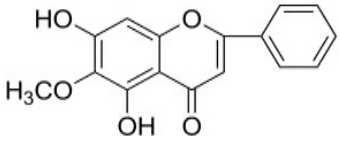
Wogonoside; mol.wt. 460



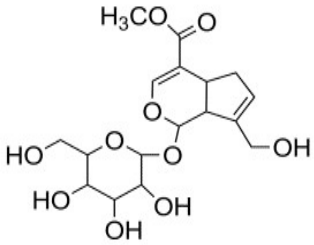
Scutellarin;mol. wt.462



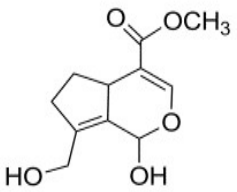
Wogonin; mol.wt. 284



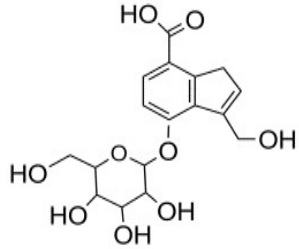
Oroxylin A; mol. wt.284



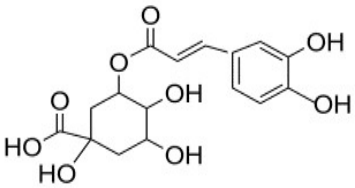
Geniposide; mol.wt. 388



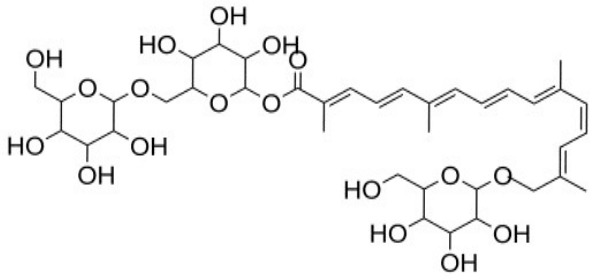
Genipin; mol.wt. 226



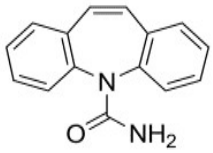
Geniposidic acid; mol.wt. 374



Chlorogenic acid;mol.wt. 354



Crocin-I; mol.wt. 976



Carbamazepine (IS); mol.wt. 236

Fig. 1: Chemical structures of 11 analytes and internal standards (IS) in HQD

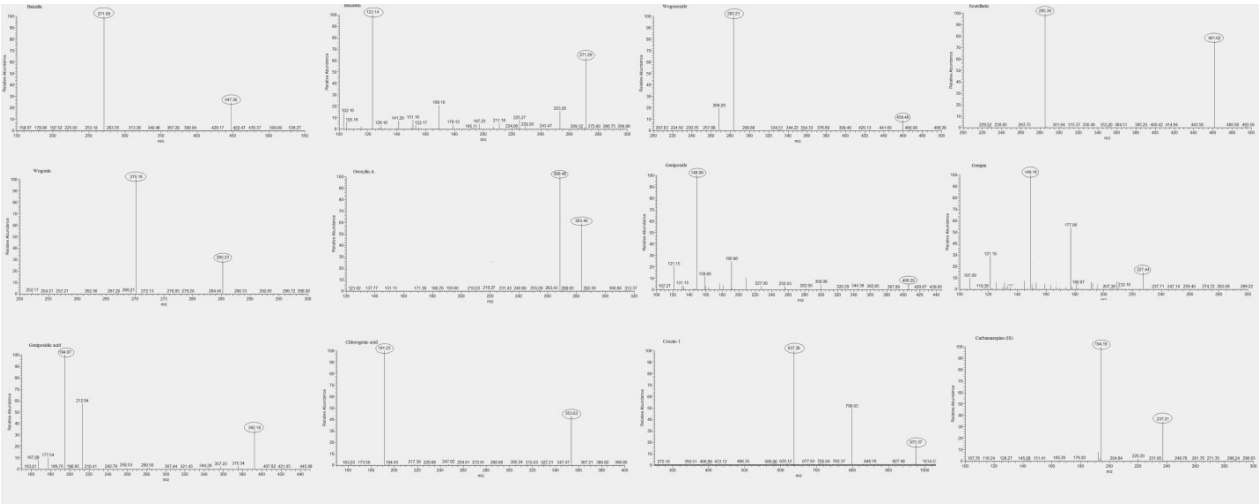


Fig. 2: Mass spectra of 11 standards and carbamazepine (IS).

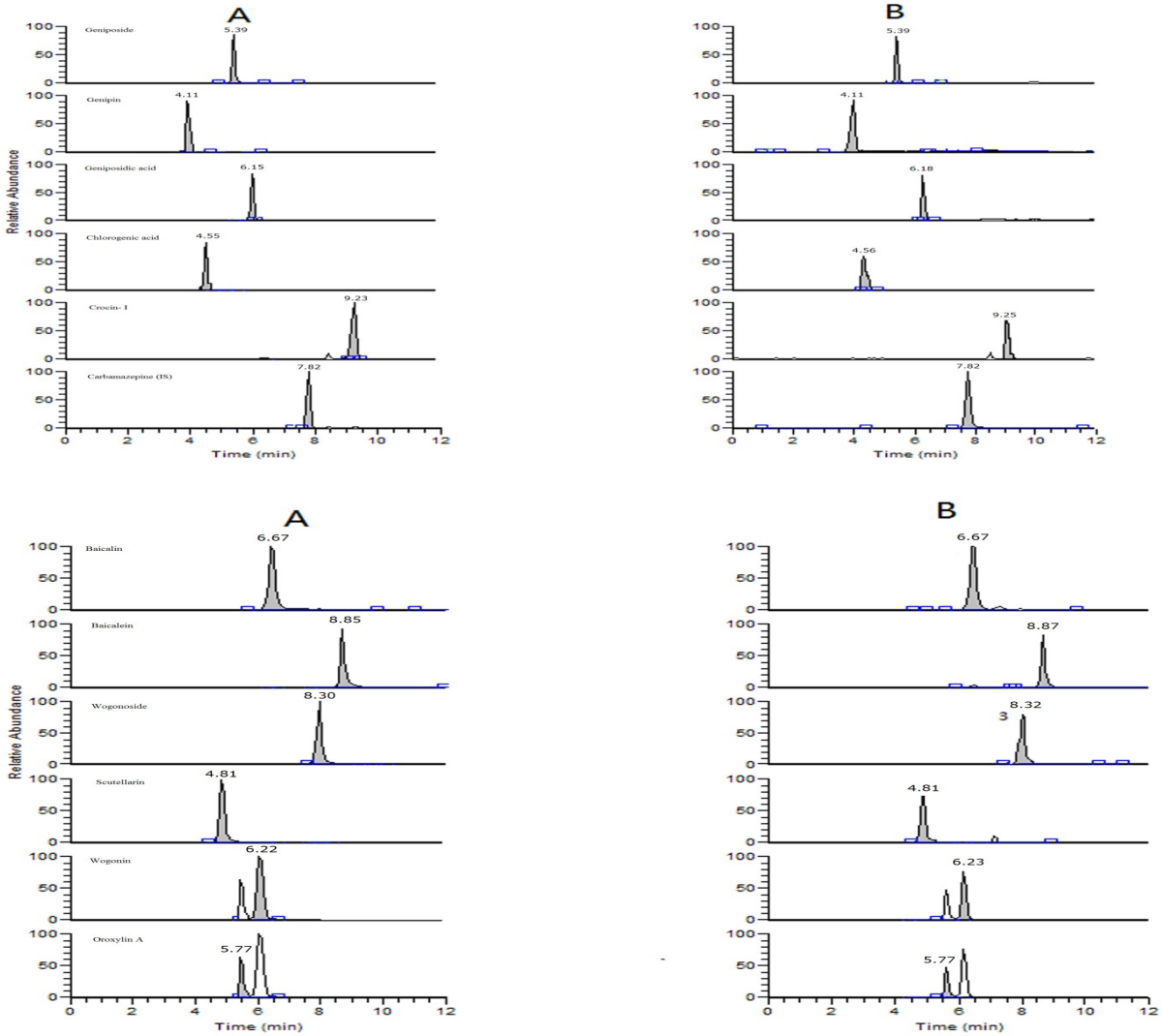


Fig. 3: SRM chromatograms of 11 analytes of (A) the reference standards and (B) HQD1 samples.

Table 1: Parameters for mass spectrometry

Analytes	*m/z*	CE(V)	Tube Lens (V)
Baicalin	447.36→271.09	26	80
Baicalein	271.29→123.14	32	45
Wogonoside	459.48→283.21	33	120
Scutellarin	461.62→285.30	25	83
Wogonin	285.23→270.18	24	60
Oroxylin A	283.46→268.48	32	55
Geniposide	406.20→148.99	25	96
Genipin	227.44→149.18	11	79
Geniposidic acid	392.18→194.97	30	69
Chlorogenic acid	353.63→191.23	20	82
Crocin- I	975.37→637.28	36	110
Carbamazepine (IS)	237.21→194.19	18	98

CE: Collison Energy

Table 2: Calibration curves, LOD and LOQ of the analytes

Analytes	Regression equation y = b + ax	R^2	Liner ranger (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Baicalin	$y = 3.61\times10^{-3} + 1.03\times10^{-3}x$	0.9997	70.00-10500.00	5.00	15.00
Baicalein	$y = 1.90\times10^{-3} + 1.32\times10^{-4}x$	0.9996	15.00-2250.00	2.44	7.32
Wogonoside	$y = 2.38\times10^{-5} + 8.63\times10^{-5}x$	0.9995	22.00-3300.00	4.14	12.42
Scutellarin	$y = 1.55\times10^{-4} + 8.73\times10^{-6}x$	0.9996	11.80-1770.00	3.93	11.80
Wogonin	$y = 0.089 + 4.23\times10^{-3}x$	0.9995	6.60-3300.00	2.20	6.60
Oroxylin A	$y = 0.051 + 3.42\times10^{-3}x$	0.9996	7.90-1185.00	2.63	7.90
Geniposide	$y = 7.42\times10^{-3} + 1.16\times10^{-4}x$	0.9996	34.00-5100.00	4.32	12.96
Genipin	$y = 6.32\times10^{-3} + 2.58\times10^{-5}x$	0.9994	8.70-855.00	1.90	5.70
Geniposidic acid	$y = 2.52\times10^{-4} + 2.98\times10^{-5}x$	0.9995	8.40-1260.00	2.79	8.40
Chlorogenic acid	$y = 1.55\times10^{-3} + 9.13\times10^{-6}x$	0.9992	10.80-2070.00	4.60	13.80
Crocin-I	$y = -2.81\times10^{-6} + 3.36\times10^{-7}x$	0.9994	7.80-1170.00	2.60	7.80

LOD: Limits of detection; LOQ: Limits of quantification

The accuracy of the analysis was estimated by intra-day and inter-day differences, which were evaluated with low, medium, and high concentrations of mixed standard solutions in the optimized conditions 6 times on day 1, and we reproduced this experiment over 3 successive days. The relative standard deviation (RSD) was used as a measurement of precision and it was detailed by relative error (RE). The spiked recovery test was utilized to assess the precision of the method. The recuperation was conducted by adding three amounts (low, middle, and high amounts) of the reference compounds to HQD samples. Three replicates were conducted for the test. The recoveries were determined by the equation: Recovery (%) = (Concentration detected - Concentration original) / Concentration spiked × 100%. To assess the reproducibility of the developed method, 6 samples of the same batch of HQD (batch no. ZY21301) were treated based on the sample preparation technique as detailed in the sample preparation section and they were examined with the determined method. The stability was verified with a sample of HQD treated via the preparation method as detailed in the sample preparation section and it was stored at 4°C and evaluated at 0, 6, 12, 24, 36 and 48h.

RESULTS

Analytical method confirmation

Linearity and sensitivity

The outcomes of the regression equation, correlation coefficient, and linear range, combined with the limit of detection (LOD) and limit of quantification (LOQ) for each of the 11 compounds are included in table 2. The correlation coefficients of every one of the calibration curves (R^2>0.9992) and the regression parameters suggested good linearity in comparatively broad concentration ranges. The LOD and LOQ were in the spans of 1.90-5.00 and 5.70-15.00ng/mL.

Precision and accuracy

The precision outcomes in table 3 reveal the intra- and inter-day RSD values of the 11 investigated constituents; they were 1.72%-5.02% and 0.63%-5.99%, respectively. The intra-day precision was 93.33%-106.74% and inter-day precision was 91.68%-107.65%.

Table 3: Precision and accuracy, recovery, repeatability and stability of analytes in HQD

Analytes	Intra-day (n = 6)		Inter-day (n = 3)		Conc. spiked (ng/mL)	Conc. original (ng/mL)	Conc. detected (ng/mL)	Recovery (n = 3)		Repeatability (n = 6)	Stability 48h (n = 6)
	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)				Mean ±SD (%)	RSD (%)	RSD (%)	RSD (%)
Baicalin	2.43	101.95	1.87	97.43	5440	6798	12016.33±234.53	95.93±4.31	4.49		
	2.95	97.83	3.06	95.31	6800	6798	13645.67±285.87	100.70±4.20	4.17	1.12	0.79
	2.36	101.1	1.15	97.19	8160	6798	15380.33±254.72	105.18±3.12	2.97		
Baicalein	4.80	98.04	4.18	95.5	1200	1495	2657.67±44.81	96.89±3.73	3.85		
	1.78	101.25	0.94	101.95	1500	1495	2953.33±73.58	97.22±4.91	5.05	2.79	3.14
	5.02	106.74	1.96	102.35	1800	1495	3257.67±38.63	97.93±2.15	2.19		
Wogonoside	3.45	100.2	4.45	95.36	1760	2123	3917.33±27.39	101.95±1.56	1.53		
	3.24	98.65	1.16	98.3	2200	2123	4299.00±48.12	98.91±2.19	2.21	3.26	2.77
	4.03	102.35	2.39	96.55	2640	2123	4816.00±67.01	102.01±2.54	2.49		
Scutellarin	4.71	101.46	3.20	101.63	944	1170	2115.67±23.69	100.18±2.51	2.51		
	2.97	97.82	1.59	98.35	1180	1170	2370.330±15.95	101.72±1.35	1.33	3.15	4.15
	4.51	101.65	1.08	100.85	1416	1170	2589.00±40.04	100.21±2.83	2.82		
Wogonin	4.67	96.04	2.88	95.76	184	217	402.57±4.82	100.85±2.62	2.60		
	3.82	99.18	1.82	98.76	230	217	437.40±8.59	95.83±3.73	3.90	3.7	3.88
	4.78	98.92	2.02	98.1	276	217	484.33±5.51	96.86±2.00	2.06		
Oroxylin A	4.15	103.09	5.99	102.18	88	204	188.90±5.12	96.48±5.82	6.03		
	1.98	101.05	1.36	101.65	110	204	211.07±3.86	97.33±3.50	3.60	4.82	5.12
	1.42	99.77	0.63	98.73	132	204	229.57±5.99	95.13±4.54	4.77		
Geniposide	4.08	101.9	3.81	107.65	2720	3334	5954.33±53.98	96.34±1.98	2.06		
	3.76	102.21	1.21	98.55	3400	3334	6665.00±73.75	97.97±2.17	2.21	4.33	3.81
	4.25	93.33	1.20	97.34	4080	3334	7521.33±31.53	102.63±0.77	0.75		
Genipin	3.41	98.05	3.05	91.68	152	189.7	334.30±8.39	95.13±5.52	5.80		
	4.57	94.93	0.98	96.55	190	189.7	377.03±6.76	98.60±3.56	3.61	2.8	3.51
	2.46	101.82	3.01	97.50	228	189.7	410.50±8.53	96.84±3.74	3.86		
Geniposidic acid	3.54	99.74	4.57	101.53	224	269.3	497.27±4.05	101.77±1.81	1.78		
	4.33	95.59	0.73	95.60	280	269.3	545.30±11.29	98.57±4.03	4.09	3.18	3.34
	3.09	104.35	3.97	105.03	336	269.3	589.27±18.66	95.23±5.55	5.83		
Chlorogenic acid	3.53	103.54	2.06	106.69	368	441.3	795.00±22.15	96.11±6.02	6.26		
	3.43	98.80	2.88	99.76	460	441.3	889.37±16.22	97.41±3.53	3.62	2.64	2.68
	3.74	96.96	1.96	96.11	552	441.3	985.63±11.86	98.61±2.15	2.18		
Crocin- I	4.47	100.14	4.19	95.73	208	253.2	448.83±7.28	94.05±3.05	3.72		
	1.72	102.41	1.55	103.18	260	253.2	504.93±6.10	96.82±2.35	2.42	2.98	3.10
	3.79	95.84	1.00	98.87	312	253.2	574.40±6.36	102.95±2.04	1.98		

SD: Standard deviation; RSD: Relative standard deviation

Table 4: Contents of 11 analytes in samples of HQD (mg/g, n=3)

Analytes	Content (mg/g)				
	HQD1	HQD2	HQD3	HQD4	HQD5
Baicalin	67.98±1.99	67.18±1.40	67.46±0.67	68.34±1.62	68.45±2.36
Baicalein	14.95±0.42	15.08±0.25	14.81±0.48	14.71±0.73	15.12±0.38
Wogonoside	21.23±0.53	21.18±0.28	21.36±0.93	21.02±0.69	21.49±0.48
Scutellarin	11.71±0.03	11.82±0.64	11.90±0.55	11.97±0.30	11.77±0.38
Wogonin	2.17±0.13	2.16±0.05	2.25±0.13	2.12±0.04	2.21±0.08
Oroxylin A	2.04±0.09	2.07±0.09	2.13±0.09	2.11±0.07	2.08±0.03
Geniposide	33.34±1.21	33.28±0.65	33.76±0.71	33.89±0.40	33.56±0.64
Genipin	1.89±0.10	1.95±0.09	1.82±0.09	1.93±0.11	1.81±0.05
Geniposidic acid	2.69±0.12	2.56±0.15	2.64±006	2.75±0.12	2.66±0.14
Chlorogenic acid	4.41±0.13	4.55±0.10	4.53±0.10	4.45±0.07	4.37±0.19
Crocin- I	2.53±0.10	2.57±0.17	2.42±0.14	2.49±0.05	2.46±0.08

Recovery, repeatability, and stability

As demonstrated in table 3, the retrieval of the 11 compounds was within 94.05%-105.18% and the RSD values were determined to be from 0.75%-6.26%. There were 6 samples of identical origins that were removed and examined with the suggested method to determine its reproducibility. The RSD values of the 11 compounds were within 1.12%-4.82%. The sample solution was established over 48h at 4°C (RSD, < 5.12%). These data suggest that the developed method is exact and explicit for the quantitation of the primary constituents in HQD.

Sample analysis

The established LC-MS/MS technique was used to determine and quantify 11 compounds in the 5 sample batches of HQD. As presented in table 4, the outcomes suggest that each one of the 11 compounds was identified in the 5 batches of HQD samples, while their contents varied substantially from every one. In the test, baicalin, geniposide, wogonoside, baicalein, and scutellarin were established as the 5 biggest concentrations components that are recognized as possessing anti-inflammatory effects (Huang, Lee *et al*., 2006, Koo, Lim *et al*., 2006, Dinda, Dinda *et al*., 2017, Fu, Liu *et al*., 2012) and function as primary active compounds in HQD. In addition, some trace but beneficial components, such as wogonin, oroxylin A, genipin, geniposidic acid, chlorogenic acid, and crocin-I, were noted and concurrently identified. The outcomes also revealed that the levels of the 11 constituents did not significantly vary in the 5 sample batches, which demonstrates the reproducibility of the HQD preparation method. Thus, the 11 compounds can be deemed to be marker compounds for quality control work on the identical herbs of botanical drugs.

DISCUSSION

Optimisation of LC-MS/MS conditions

To acquire a more intense peak and more sensitive signal reaction, methanol-water and acetonitrile-water were evaluated as the mobile phase compositions. Further, acid formic and acetic acid were chosen as the acidic additive of the mobile phases, and ammonium acetate and ammonium formate were added to the mobile phase with different volume contents to attain a high sensitivity and they were contrasted. The optimal mobile phase, with methanol and water (0.1% formic acid with 1mmol/L ammonium acetate), was at last used with a flow of 0.3mL/min in the above gradient and it generated a high-resolution and symmetrical peak.

Taking into account optimum ionization efficiency and maximal sensitivity, the heated ESI source was used with polarity switching among positive and negative ion modes with selected reaction monitoring (SRM) in a single run. The mass spectral conditions were optimized with the reference compounds. In the negative ionization mode, deprotonated $[M-H]^-$ molecular ions of wogonoside, scutellarin, oroxylin A, chlorogenic acid and crocin-I were plentifully produced, and the 6 other compounds revealed protonated $[M+H]^+$ (baicalin, baicalein, wogonin, and genipin) or $[M+NH_4]^+$ (geniposide and geniposidic acid) molecular ions in the positive ionization mode. Via manual optimization, utilizing infusion with a syringe pump, the appropriate heated capillary temperature, spray voltage, collision energy and tube lens with SRM for every one of the 11 target analytes and the IS were identified. The in-depth mass spectrometer data and SRM chromatograms are revealed in table 1 and figs. 2-3.

Optimization of the sample extraction method

Extraction method, extraction solvent and extraction time were examined in order to optimize the extraction procedure. For the extraction methods, ultrasonication and heat-reflux were examined and the outcomes showed that ultrasonic extraction was more efficient and advantageous. Various kinds of solvent (methanol or ethanol) at varying ratios (60%, 80% and 100%, v /v) with the solvent volume (50, 100 and 200mL) and the extraction time (15, 30 and 60 min) were also evaluated. The outcomes revealed that the most satisfactory conditions for

extraction were acquired using 1.0g of the sample in powder form removed with 100mL of 80% methanol in an ultrasonic bath for 30 min.

CONCLUSION

The traditional LC-MS/MS technique was appropriate and dependable for the quantitative evaluation of the primary parts of HQD. The technique effectively quantified 11 compounds in 5 samples of HQD and showed that the contents were steady. The validation outcomes revealed that the suggested technique is a dependable and sensitive quality assessment method for HQD. It may be utilized to regulate the quality of HQD.

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