

# Simultaneous Determination of Ten Constituents in Chaikin Qingning Capsule by High-performance Liquid Chromatography Coupled with Triple-quadrupole Mass Spectrometry

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## ABSTRACT

**Background:** Chaikin Qingning Capsule (CQQNC) was a prescription of Traditional Chinese Medicine with the effects of clearing away heat and removing toxin, harmonizing the exterior and interior, it was widely used in Asian, for example, China and Japan, different batches of the raws materials and different processing time may be the vital factor which raised a challenge to control the quality of the CQQNC.

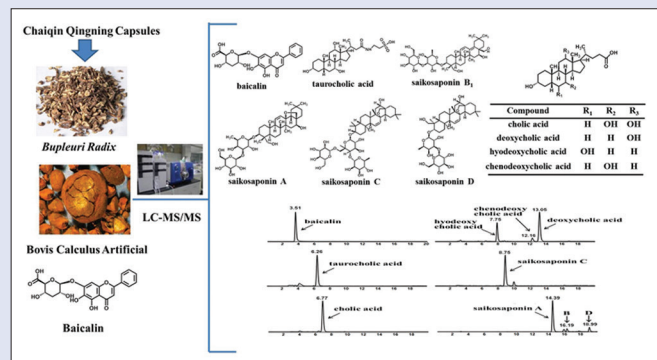
**Experimental Methods:** In this experiment, a high-performance liquid chromatography-mass spectrometry/MS (HPLC-MS/MS) method was developed to simultaneously determine ten bioactive components for the quality control of CQQNC. Chromatographic separation was achieved using an XBridge BEH C18 column (150 mm × 4.6 mm, 2.5 μm) with a mobile phase composed of 10 mM aqueous ammonium acetate and acetonitrile using a gradient elution in 20 min. This study was conducted by multiple reaction monitoring mode through electrospray ionization resource with a negative ionization mode. **Results:** The established method was validated with good performance of precision, accuracy, stability, and reproducibility and was utilized to simultaneously quantify ten constituents of CQQNC obtained from seven different batches.

**Conclusion:** It is the first time to report the rapid and simultaneous analysis of the ten compounds in CQQNC by HPLC-MS/MS and apply to determine 10 constituents in 7 batches of CQQNC bought from drug store in china. This method could be considered as good quality criteria to control the quality of CQQNC.

**Key words:** Chaikin Qingning Capsule, content determination, high-performance liquid chromatography-mass spectrometry

## SUMMARY

- In this paper, a simple, specific, and rapid high-performance liquid chromatogram coupled with triple-quadrupole mass spectrometry method for simultaneous quantification of ten constituents in Chaikin Qingning Capsule has been developed for the first time. This method could be considered as good quality criteria to control the quality of CQQNC.



**Abbreviations used:** CHM: Chinese herbal medicine; TCM: Traditional Chinese Medicine; CQQNC: Triple-quadrupole mass spectrometry Chaikin Qingning Capsules; HPLC-MS/MS: High liquid chromatography equipped with tandem mass spectrometry; ESI: Electrospray ionization; DP: Declustering potential; CE: Collision energy; RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantity.

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## INTRODUCTION

The prescription of Chinese herbal medicine (CHM) is developed basing on the theories of Traditional Chinese Medicine (TCM). CHM, a significant part of Chinese culture, has its distinguished methodology, epistemology, and systematic theory. Its experience has accumulated for a long history of more than 1000 years, and thus, it has its unique diagnosis and treatment system.<sup>[1]</sup> A series of CHMs has passed through clinical certification of the American Food and Drug Administration (FDA). For example, compound danshen dripping pills, guizhi fuling capsule,

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Fuzheng Huayu tablets, and so on.<sup>[2]</sup> Overall, CHM has been received more and more attention and widely used all over the world.

Chaiqin Qingning Capsules (CQQNCs) is a kind of CHM, which was consist of baicalin, Bupleuri Radix (roots of *Bupleurum chinense* DC.) and calculus bovis factitious. CQQNCs has the efficacy of clearing away heat and removing toxin, harmonizing the exterior and interior, which was widely used to treat evil of respiratory tract infection in lung health symptom, such as fever with cold aversion, pharyngalgia, and turbid nasal discharge. As a TCM formula, CQQNC has the advantage of high safety and small side effects, which also could avoid the risk of hepatic failure caused by acetaminophen overdose, and the phenomenon of antibacterial drug abuse.

In our study, for the first time, a quick, sensitive, and precise analytical method high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) was used to measure 10 compounds in CQQNC at the same time: baicalin (1), cholic acid (2), taurocholic acid (3), deoxycholic acid (4), hyodeoxycholic acid (5), chenodeoxycholic acid (6), saikosaponin A (7), saikosaponin B1 (8), saikosaponin C (9), and saikosaponin D (10). Validation parameters of the analytical method have been determined systemically, including susceptibility, accuracy, and linear range. The applied method was used to successfully quantify 10 components at the same time in seven batches of CQQNC purchased from home markets.

## EXPERIMENTAL METHODS

### Materials and reagents

The formic acid (Dikma Technologies Company, California, USA), acetonitrile (Tedia Company Inc., Fairfield, OH, USA),

and methanol (Concord Technology Company, China) meet HPLC requirements. Using an ultrapure Milli-Q Academic water system (Millipore, Milford, MA, USA), deionized water was purified. All other reagents were of analytical grade and purchased from Tianjin Concord Technology Co. Ltd. (Tianjin, China). The chemical structures of baicalin, cholic acid, taurocholic acid, deoxycholic acid, hyodeoxycholic acid, chenodeoxycholic acid, saikosaponin A, saikosaponin B1, saikosaponin C, and saikosaponin D are shown in Figure 1. The reference substances (purity  $\geq 98\%$ ) in the study using for quantitative analysis were bought from Sichuan Pufeide Biological Technology Co., Ltd in China. Seven batches of CQQNC (batch No. 15020741, 12031911, 12032011, 12100911, 12101012, 12101011, and 12031811) were provided by Yangtze River Pharmaceutical Group Co., Ltd. (Taizhou, Jiangsu, China) and deposited at key laboratory of drug metabolism and disposition, Dalian Medical University.

### Preparation of standard solutions

Primary stock solutions of baicalin, cholic acid, taurocholic acid, deoxycholic acid, hyodeoxycholic acid, chenodeoxycholic acid, saikosaponin A, saikosaponin B1, saikosaponin C, and saikosaponin D were prepared separately at a final absorption of 0.10 mg/mL, respectively, by melt accurately weighed reference compounds in methanol. Working solutions were achieved by mitigated the stored ones in acetonitrile or water (65:35, v/v) at concentration between 313 and 10,000 ng/mL each baicalin; 31.3–1000 ng/mL for cholic acid, taurocholic acid, hyodeoxycholic

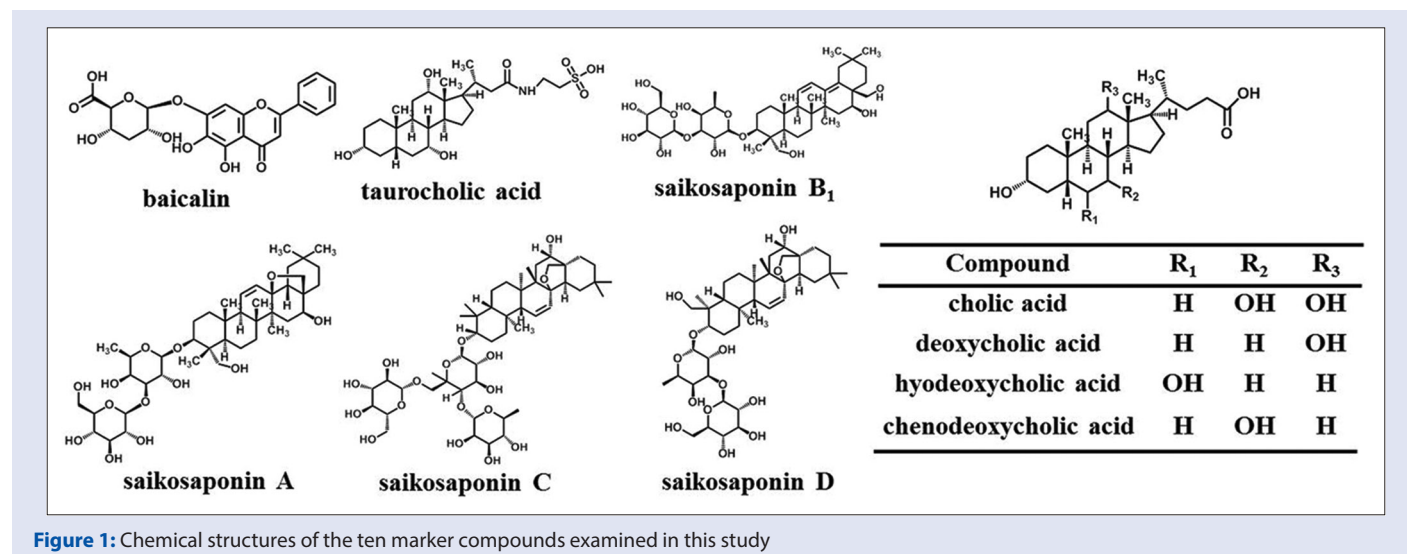


Figure 1: Chemical structures of the ten marker compounds examined in this study

Table 1: Multiple reaction monitoring parameters, decluttering potential, and collision energy values for the ten analytes

Analyte	Monitoring ion	Precursor ion (m/z)	Production (m/z)	DP (V)	CE (eV)
Baicalin	[M-H] <sup>-</sup>	445.0	268.9 <sup>a</sup>	-80	-29
Cholic acid	[M-H] <sup>-</sup>	407.2	407.2 <sup>a</sup> , 343.1, 289.3	-135	-15
Taurocholic acid	[M-H] <sup>-</sup>	514.0	514.0 <sup>a</sup>	-135	-15
Deoxycholic acid	[M-H] <sup>-</sup>	391.1	391.1 <sup>a</sup> , 345.1	-135	-15
Hyodeoxycholic acid	[M-H] <sup>-</sup>	391.1	391.1 <sup>a</sup> , 373.1	-135	-15
Chenodeoxycholic acid	[M-H] <sup>-</sup>	391.1	391.1 <sup>a</sup> , 373.0	-135	-15
Saikosaponin A	[M-H] <sup>-</sup>	779.1	779.1 <sup>a</sup> , 617.2	-220	-15
Saikosaponin B1	[M-H] <sup>-</sup>	779.1	779.1 <sup>a</sup> , 617.0	-220	-15
Saikosaponin C	[M-H] <sup>-</sup>	925.3	925.3 <sup>a</sup> , 779.2, 617.1	-220	-15
Saikosaponin D	[M-H] <sup>-</sup>	779.1	779.1 <sup>a</sup> , 617.1	-220	-15

<sup>a</sup>The product ion selected for quantitative MRM. MRM: Multiple reaction monitoring; DP: Decluttering potential; CE: Collision energy

acid and saikosaponin A, 15.6–500 ng/mL for deoxycholic acid, 6.25–200 ng/mL for chenodeoxycholic acid, and 3.13–100 ng/mL for saikosaponin B1, saikosaponin C, and saikosaponin D. All of them were deposited at 4°C.

### Preparation of sample solutions

The content was taken from three capsules and ground to fine powder. We transfer 10 mg precisely evaluated powder into a 50 mL Teflon-lined container of volumetric flask and added 50% methanol. The drawing solution was extracted at 60°C for 45 min. The resulting extract solution was centrifuged for 10 min at 2000 g, filtered by a 0.22 µm millipore filter. Finally, 10 µL samples were put into the HPLC-MS/MS system.

### High-performance liquid chromatography-mass spectrometry analysis

With a Shimadzu LC-2010AHT system (Shimadzu, Kyoto, Japan), HPLC analysis were carried out in a column department and a temperature-controlled autosampler keep at 4°C. Chromatographic analysis was obtained by applying an XBridge BEH C18 column (150 mm × 4.6 mm, 2.5 µm) at 30°C. The mobile phase combined with 10 mm ammonium acetate (A) and acetonitrile (B), the flow ratio was set to 0.5 mL/min. The gradient program was 35% B from 0 to 3 min, 35%–40% B from 3 to 9 min, 40%–55% B from 9 to 14 min, 55% B from 14 to 17 min, and 35% B from 17 to 20 min. Ten microliter samples were applied with the HPLC-MS/MS for the study.

**Table 2:** Linear regression with *r* value and linear range, retention time, the limit of detection, and limit of quantity value for the ten analytes

Compound	Linear equation	<i>r</i>	Range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	<i>t<sub>R</sub></i> (min)
Baicalin	$Y=1.16 \times 10^6 X + 2.29 \times 10^5$	0.9991	313-10,000	10.78	36.17	3.55
Cholic acid	$Y=7.6 \times 10^6 X + 8.9 \times 10^4$	0.9989	31.3-1000	1.95	5.11	6.63
Taurocholic acid	$Y=1.62 \times 10^6 X + 1.35 \times 10^4$	0.9982	31.3-1000	0.45	1.56	6.28
Deoxycholic acid	$Y=1.03 \times 10^4 X + 2.98 \times 10^4$	0.9994	15.6-500	0.52	1.59	12.7
Hyodeoxycholic acid	$Y=3.55 \times 10^6 X + 1.69 \times 10^4$	0.9990	31.3-1000	0.49	1.30	7.66
Chenodeoxycholic acid	$Y=2.60 \times 10^3 X + 4.84 \times 10^3$	0.9998	6.25-200	0.78	1.95	11.9
Saikosaponin A	$Y=2.53 \times 10^6 X + 1.70 \times 10^4$	0.9994	31.3-1000	0.49	1.66	14.4
Saikosaponin B1	$Y=2.12 \times 10^3 X + 901$	0.9960	3.13-100	1.56	3.93	16.2
Saikosaponin C	$Y=1.67 \times 10^3 X + 1.64 \times 10^3$	0.9990	3.13-100	0.78	1.86	8.73
Saikosaponin D	$Y=2.84 \times 10^3 X + 4.39 \times 10^3$	0.9990	3.13-100	0.78	2.56	19.0

Y: Peak area; X: Concentration of the standard (µg/mL); *r*: Correlation coefficient of the equation; LOD: Limit of detection; LOQ: Limit of quantification; *t<sub>R</sub>*: Retention time

**Table 3:** Accuracy, reproducibility, stability, and recovery of the ten analytes

Compound	Precision (RSD%)		Reproducibility (RSD%, <i>n</i> =6)	Stability (RSD%, <i>n</i> =6)	Recovery (%; <i>n</i> =3)				
	Intraday ( <i>n</i> =6)	Interday ( <i>n</i> =3)			Original (µg)	Spiked (µg)	Found (µg)	Recovery (%)	RSD (%)
Baicalin	2.28	0.46	3.58	3.62	3325	1663	4933	96.7	0.65
					3325	6680	100.9	3.81	
					4988	8408	101.9	0.34	
Cholic acid	1.37	0.20	2.73	4.32	195	97	299	102.4	3.10
					195	402	101.3	1.06	
					292	489	101.0	0.30	
Taurocholic acid	4.18	0.85	1.94	2.85	138	69	199	97.7	2.10
					138	294	101.0	0.72	
					208	342	98.0	1.12	
Deoxycholic acid	0.34	1.71	2.44	2.66	64.6	32.3	98.9	101.0	2.36
					64.6	133	100.1	0.64	
					96.8	162	100.9	1.88	
Hyodeoxycholic acid	1.59	2.30	1.62	1.41	99	49	153	102.6	1.13
					99	202	98.4	1.75	
					148	248	100.7	1.71	
Chenodeoxycholic acid	4.65	3.76	2.60	2.08	29.6	14.8	46.2	101.3	0.17
					29.6	61.5	98.2	0.22	
					44.4	73.9	99.9	4.09	
Saikosaponin A	1.21	0.92	0.74	1.69	126	63	192	102.5	2.63
					126	253	101.1	1.68	
					189	315	100.2	1.59	
Saikosaponin B1	3.50	2.74	3.33	3.16	11.8	5.9	18.3	98.9	0.31
					11.8	23.8	101.1	0.56	
					17.7	29.9	101.6	2.94	
Saikosaponin C	4.94	1.48	4.24	3.13	17.6	8.8	27.5	101.3	1.45
					17.6	37.2	100.2	0.76	
					26.4	44.8	102.8	1.48	
Saikosaponin D	2.46	2.26	3.72	4.09	15.0	7.5	22.9	102.2	1.39
					15.0	30.4	102.6	0.22	
					22.5	37.4	99.8	1.11	

RSD: Relative standard deviation

MS/MS (triple-quadrupole tandem mass spectrometry) was performed on an AB SciexQTrap<sup>®</sup> 4500 mass spectrometer (AB Corp.) with an electrospray ionization (ESI) interface. The ESI resource was conducted in negative ionization method. Quantification was operated in multiple reaction monitoring (MRM) method. The MS parameters were these: ion spray voltage, capillary, 3.5–4.0 kV; resource temperature, 120°C; and turbo desolvation temperature, 500°C. Nitrogen was applied, respectively, as the desolvation and cone gas at a flow ratio of four hundreds and 30 L each hour. Argon was operated as the collision gas. Gas source 1, 30; gas source 2, 40; collision gas, medium; curtain gas, 12. The precursor-to-generate ion pairs, declustering potential (DP), and collision energy (CE) of these analytes are showed in Table 1. All statistics was accumulated with the centroid mode and were obtained and developed with Analyst<sup>®</sup> 1.6.2 Software (applied biosystems).

## Method validation

The calibration curve was achieved by diluting the working solution of each standard to 6 proper concentrations in triplicate; the chromatographic peak area was set as y axis against the matching concentration as X axis. The limit of quantity (LOQ) and the limit of detection (LOD) were, respectively, setup as indicators to noise rate of 3 and 10.

Intra- and inter-day variations were determined as signal of the accuracy of the advanced system, and they were measured by choosing the 10 analytes in 6 replicates within a day, and then, duplicating the experiments on the following 3 days. Relative standard deviation (RSD %) means variation in the peak area. Precise was settled basing on the amount of each recovered compound. Sample 1 (reference recipe, batch no. 15020741) was added with 3 kinds (high, middle, and low) of the ten reference compounds, average recovery was measured by applying this formula: recovery (%) = (observed amount – original amount)/spiked amount × 100%, and RSD (%) = (standard deviation/mean) × 100%.

Six separated solutions of sample 1 (batch no. 15020741) were analyzed in reproducibility tests, and RSD was thought to be the standard measure. To study analytes stability, six-separated solutions of sample one (batch no. 15020741) were analyzed, respectively, at 0, 2, 4, 8, 12, and 24 h after deposit at 4°C.

## RESULTS AND DISCUSSION

### High-performance liquid chromatography-mass spectrometry optimization

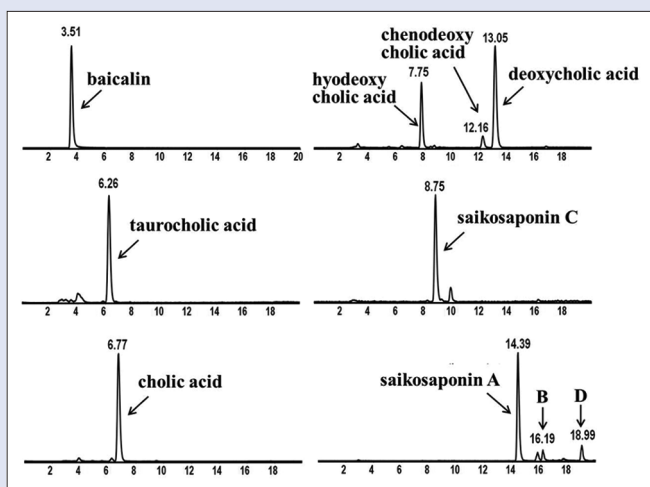
To get a higher sensitivity and better separation, some HPLC parameters were optimized. Better separation was obtained using acetonitrile instead of methanol as the organic phase. What's more, various kinds and concentrations of eluent additives were tested, including acetonitrile-water, acetonitrile-aqueous with 0.01% formic acid, acetonitrile-aqueous with 10 mm ammonium acetate, and the one with 0.1% formic acid, the result show that water with 10 mm ammonium acetate has a better peak shape and a higher resolution. Using an XBridge BEH C18 column (150 mm × 4.6 mm, 2.5 μm) showed best separation compared with Hypersil BDS-C18 column (4.6 mm × 150 mm, 5 μm) and the ZORBAX Extend-C18 column (100 mm × 2.10 mm, 3.5 μm). The column temperature was 30°C at a flow ratio of 0.5 mL/min to make sure good separation.

The reference compounds were used to optimize the MS scan mode condition. In the negative ion mode, high sensitivity and clear mass range were obtained, the quasi-molecular ions [M-H]<sup>-</sup> of ten references were produced, and their generated ions were high and with excellent specificity. With the purpose to optimize the detection and separation of

**Table 4:** Content of the ten analytes in Chaqin Qingning Capsule samples<sup>a</sup> (n=3)

Batch number	Contents (mg/g)									
	Baicalin	Cholic acid	Taurocholic acid	Deoxycholic acid	Hyodeoxycholic acid	Chenodeoxycholic acid	Saikosaponin A	Saikosaponin B1	Saikosaponin C	Saikosaponin D
15020741	303±3.9	19.3±0.96	11.5±0.18	4.02±0.09	7.71±0.21	3.93±0.13	7.67±0.19	0.50±0.02	1.51±0.07	5.64±0.14
12031911	247±6.0	15.4±0.17	10.1±0.35	5.10±0.23	8.28±0.20	2.42±0.04	8.75±0.36	1.49±0.05	1.43±0.06	2.41±0.05
12032011	247±4.3	16.6±0.80	10.8±0.53	5.62±0.18	8.90±0.12	2.67±0.06	9.51±0.10	1.51±0.02	1.45±0.02	2.39±0.10
12100911	310±3.6	17.6±0.23	10.7±0.18	5.77±0.20	9.63±0.37	2.73±0.10	5.15±0.17	1.13±0.02	1.34±0.05	1.23±0.03
12101012	319±6.7	17.0±0.48	10.6±0.42	5.52±0.06	9.38±0.25	2.64±0.06	10.9±0.24	1.04±0.02	1.49±0.04	1.55±0.08
12101011	259±3.9	16.8±0.26	10.9±0.18	5.30±0.14	9.49±0.30	2.54±0.12	8.05±0.14	1.02±0.02	1.21±0.02	1.73±0.03
12031811	253±5.9	14.3±0.13	8.7±0.13	5.33±0.09	7.33±0.23	2.19±0.10	7.60±0.29	0.82±0.03	1.49±0.04	2.28±0.01

<sup>a</sup>Contents: Mean±SD. SD: Standard deviation



**Figure 2:** Representative multiple reaction monitoring chromatograms for ten components in Chaiqin Qingning Capsules

the various compounds in CQQNC and reduce the required analytical time, other MS parameters were investigated, such as DP (–80, –135 and –220) and CE (–15, –29 and –50 eV), all of which was in Table 1. MRM extracted ion chromatograms of the reference standards were displayed in Figure 2.

### Validation results

The regression equation, LOD, LOQ values, and linear dynamic ranges of all the reference compounds were showed in Table 2. Ten compounds demonstrated a satisfactory linearity ( $r > 0.9960$ ) above a suitable concentration scale. The LOD and LOQ values of ten compounds were ranged from 1.30 to 6.17 ng/mL and 0.45–10.78 ng/mL. The RSD values of intra- and inter-day precisions were  $< 4.94\%$ . The recovery was ranged from 96.7% to 102.8% with RSD values equal or  $< 4.09\%$  [Table 3]. The RSD values of all analyses were  $< 4.24\%$  in the reproducibility test. The samples stored at 4°C were stable for 2 h with RSD  $< 4.32\%$ . These findings show that this method with high accuracy and sensitivity is proper for determining these ten compounds in CQQNC samples at the same time.

### Sample analyses

The validated HPLC-MS/MS method was utilized to simultaneously quantify ten constituents of CQQNC from seven different batches. The content of compounds was shown in Table 4. There were many differences in the content of the components between seven batches. For example, the contents of baicalin and saikosaponin B1 differed between 4 and 3 times, respectively, which may result in the change of drug efficacy. It should be paid more attention in clinical application.

In this report, a HPLC-MS/MS method for simultaneous qualification of 10 components in CQQNC was developed. First of all, this is the

first report about quality control of CQQNC. According to the State FDA of China, baicalin was chosen as index component of CQQNC for quality control, but as a compound preparation, multi-index components determination which was used to chemically characterize the similarity and distinctness between the different batches, serving for their quality control, and overall assessment were necessary.<sup>[3,4]</sup> Second, two groups compound with similar structure and similar molecular weight were obtained good separation, which including one group of cholic acid, taurocholic acid, deoxycholic acid, hyodeoxycholic acid and chenodeoxycholic acid, another group of saikosaponin A, saikosaponin B1, saikosaponin C, and saikosaponin D. Third, during the specific and sensitive ESI-MS/MS detection, MRM method was used to exclude false-positive finding. Fourth, the values of LOD and LOQ under our method were less than the compounds in other references.<sup>[5,6]</sup>

### CONCLUSION

For the first time, a sensitive and fast analytical method was developed using HPLC-MS/MS for the quantitative and qualitative of 10 bioactive compounds in CQQNC. The established method was also applied to determine their contents in seven batches, which provide great support for better quality control and establishment of CQQNC.

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### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

1. Lei X, Chen J, Liu CX, Lin J, Lou J, Shang HC, *et al.* Status and thoughts of Chinese patent medicines seeking approval in the US market. *Chin J Integr Med* 2014;20:403-8.
2. Ye XF, He J. The bright future of Chinese herbal medicine: Only after a twisty road. *Contemp Clin Trials* 2010;31:508-9.
3. Zheng L, Huo XK, Wang C, Cong HJ, Xiang T, Wu B, *et al.* Simultaneous determination of 14 components in Shuanghua Baihe tablets by ultrasonic-assisted extraction and HPLC-MS/MS. *Anal Methods* 2016;8:3359-65.
4. Xu HY, Xiang H, Zhan LB, Zhang L, Sun, XX. Simultaneous determination of eight ginsenosides in Zibu Pi Yin recipe using ultra-performance liquid chromatography coupled with triple-quadrupole mass spectrometry (UPLC-MS/MS). *Anal Methods* 2015;7:7527-33.
5. Chen D, Lin S, Xu W, Huang M, Chu J, Xiao F, *et al.* Qualitative and quantitative analysis of the major constituents in Shexiang Tongxin dropping pill by HPLC-Q-TOF-MS/MS and UPLC-QqQ-MS/MS. *Molecules* 2015;20:18597-619.
6. Yang HJ, Ma JY, Weon JB, Lee B, Ma CJ. Qualitative and quantitative simultaneous determination of six marker compounds in Soshiho-tang by HPLC-DAD-ESI-MS. *Arch Pharm Res* 2012;35:1785-91.