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Short communication

# Simultaneous determination of 11 active components in two well-known traditional Chinese medicines by HPLC coupled with diode array detection for quality control

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ABSTRACT

A simple and sensitive high-performance liquid chromatography coupled with diode array detection (HPLC-DAD) method was investigated for simultaneous determination of 11 components (chlorogenic acid, coptisine, epiberberine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and chrysin) in *Qinhuanghouzheng* (QHHZ) capsule and *Xiaoerqingre* (XEQR) tablet, for quality control of these two well-known traditional Chinese medicines (TCMs). The method was established using an Eclipse Plus $C_{18}$ (150 mm × 4.6 mm i.d., 5 μm) column. The mobile phase comprising methanol (A) 3% phosphoric acid (B) (pH 2.0, adjusted by triethylamine) was used to elute the targets in gradient elution mode. Flow rate and detection wavelength were set at 0.8 mL/min and 270 nm, respectively. All calibration curves showed good linearity with $R^2 > 0.9995$. Inter- and intra-day precisions for all investigated components expressed as relative standard deviation (R.S.D.) ranged from 0.26% to 1.77%. Recoveries measured at three concentrations were in the range of 95.0–103.0% with R.S.D. ≤ 3%. The validated method is simple, reliable, and successfully applied to determine the contents of the selected compounds in QHHZ capsule and XEQR tablet for quality evaluation and control. The 11 main active marker compounds measured occur only in 2 or 3 plant species out of 7–10 species comprising the two TCMs. Additional procedures need to be developed for the quality control of plant materials other than *Coptis chinensis* Franch, *Scutellaria baicalensis* Georgi and *Phellodendron amurense* Rupr.



## 1. Introduction

Traditional Chinese medicines (TCMs) have a long history, and have an indispensable role in the healthcare system in China. They have been the major foundation for the prevention and treatment of many diseases for centuries [1]. Increasing attention is being paid to TCMs for the research and development of new drugs. Controlling and evaluating the quality of herbal products is very important for their safety and efficacy. Research into quality control of TCMs is therefore warranted.

In recent years, many analytical techniques have been developed for evaluating the quality of herbs or herbal preparations. These include determination of single compound or multiple constituents, as well as fingerprint analysis. Of these, single marker compound determination is simple, but it cannot afford sufficient quantitative information for other active components in complex TCMs [2–4]. Fingerprint analysis can evaluate the quality consistency and stability of herbal products, but cannot enable accurate quantification of analytes [5–7]. Many pure standards are required and suitable chromatographic conditions are difficult to optimize, but multi-constituent determination is widely used to control the quality of TCMs [8–10] because of the advantage of simultaneous determination of many markers from different herbs for evaluation of total quality. In the process, technologies such as high-performance liquid chromatography (HPLC) [11–13], high-performance capillary electrophoresis (HPCE) [14–16], and liquid chromatography–mass spectrometry (LC–MS) [17–19] are often used. HPLC is simple, sensitive and inexpensive, and has been widely used in the pharmaceutical field.

The herbal preparations *Qinhuanghouzheng* (QHHZ) capsule and *Xiaoerqingre* (XEQR) tablet have been widely used in China. The former is used for the treatment of pharyngitis [20]. It is composed of seven medicinal herbs: *Coptis chinensis* Francs; *Scutellaria baicalensis* Georgi; *Gardenia jasminoides* Ellis; *Radix et Rhizoma Rhei*; *Curcuma aromatica* Salisb *Borneol*; and *Artificial bezoar*. The later, used for the treatment of pedo-windheat, fantod, hyperspasmia

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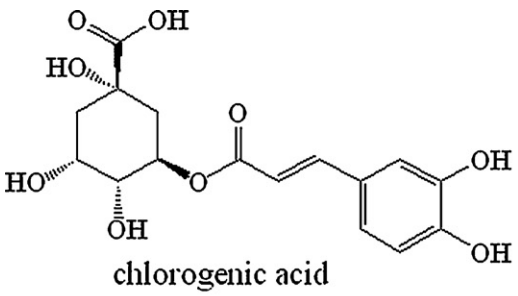


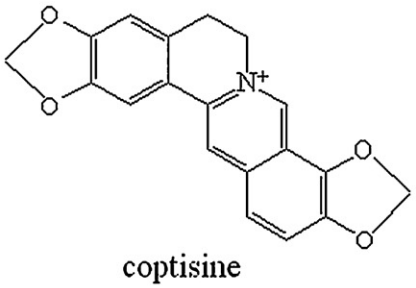


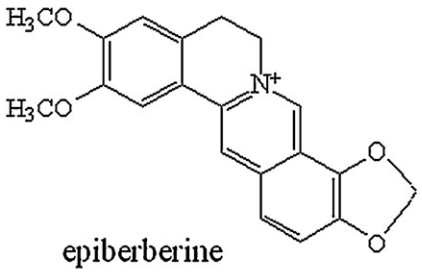


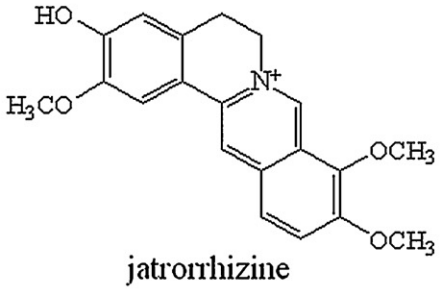


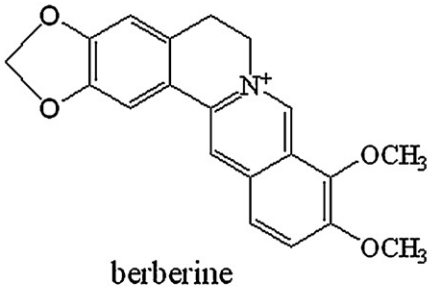


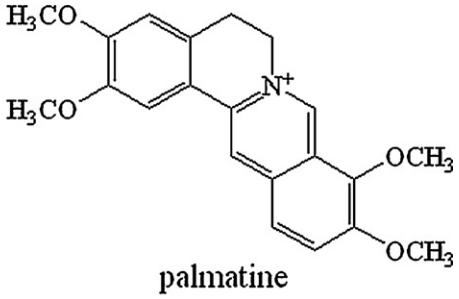


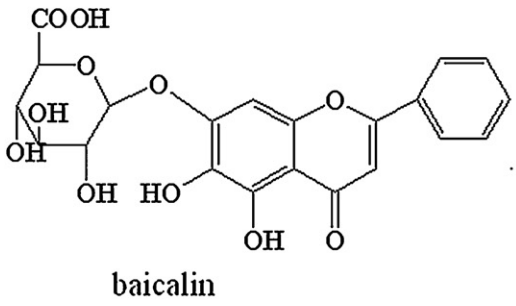


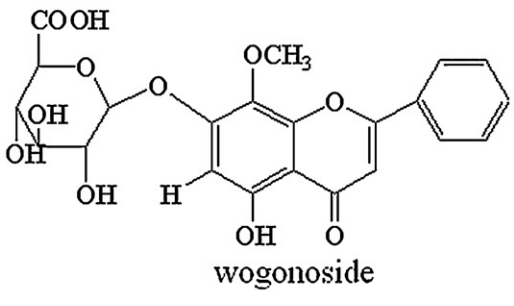


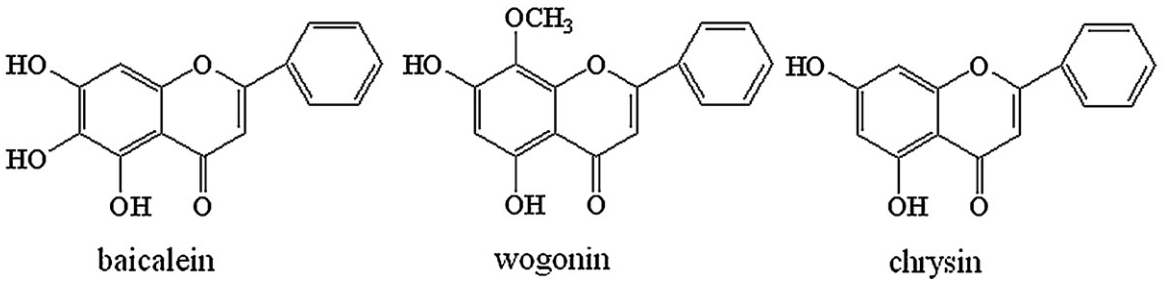


**Fig. 1.** The chemical structures of the tested components.

and febrile canker sore [21], is composed of: *C. chinensis* Franch; *S. baicalensis* Georgi; *Phellodendron amurense* Rupr; *G. jasminoides* Ellis; *Radix et Rhizoma Rhei*; *Radix gentianae*; *Uncaria macrophylla* Wall; *Cinnabar*; *Realgar*; *Cinnabaris*; *Peppermint oil*; and *Juncus effusus* L. Chemical and pharmacological studies have shown that flavonoids including baicalin, wogonoside, baicalein, wogonin and chrysin [22–24] from *S. baicalensis* Georgi, and alkaloids including coptisine, epiberberine, jatrorrhizine, berberine and palmatine [25]

from *C. chinensis* Franch or *P. amurense* Rupr, are considered to be the active compounds from QHHZ capsule and XEQR tablet. These components are usually regarded as the markers of quality control and evaluation only by consideration of their actions, contents and strong UV absorptions. We cannot find articles reporting the quality control of the two TCMs in the literature.

A convenient, reliable and sensitive HPLC method for simultaneous determination of chlorogenic acid, coptisine, epiberberine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and chrysin (Fig. 1) in two well-known TCMs, QHHZ capsule and XEQR tablet, was developed. This is the first report of the simultaneous determination of the 11 compounds in QHHZ capsule and XEQR tablet.

## 2. Experimental

### 2.1. Chemicals and reagents

Methanol was of HPLC grade (Tedia Company Inc., Fairfield, OH, USA). Triethylamine, phosphoric acid and other regents were of analytical grade and purchased from ShenLian Chemical Factory (Dalian, China). Reverse osmosis Milli-Q water (18 MΩ; Millipore, USA) was used for all solutions and dilutions. Reference standards of coptisine and epiberberine were isolated in our laboratory with purities of >98%, and structures were identified by ultraviolet (UV) absorption, mass spectrometry (MS), nuclear magnetic resonance (NMR) and confirmed by the literatures [25,26]. Other standards, including chlorogenic acid, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and chrysin, were purchased from the Chinese National Institute of Control of Pharmaceutical and Biological Products, Beijing, China.

Twenty-two batches of QHHZ capsules and three batches of XEQR tablets were obtained from six and two manufacturers, respectively, in China. The information of the samples is listed in Table 1. The medicinal herbs *C. chinensis* Franch, *S. baicalensis* Georgi, *P. amurense* Rupr, *G. jasminoides* Ellis, *Radix et Rhizoma Rhei*, *R. gentianae*, *U. macrophylla* Wall, *Cinnabar*, *Realgar*, *Cinnabaris*, *Peppermint oil*, *C. aromatica* Salisb, *Borneol*, *Artificial bezoar*, and *J. effusus* L. were purchased from a local drug store (Dalian, China) and authenticated by Dr. Yunpeng Diao (Dalian Medical University, Dalian, China).

### 2.2. Preparation of standard solutions

Standard stock solutions of the 11 reference standards (chlorogenic acid, coptisine, epiberberine, jatrorrhizine, berberine, palmatine baicalin, wogonoside, baicalein, wogonin and chrysin) were prepared by dissolving them in methanol. They were then diluted to seven concentrations for construction of calibration plots in the ranges of 1.56–184, 6.75–540, 15–480, 12.75–510, 25–850, 12.5–600, 1.56–750, 1.625–120, 0.625–40, 0.78–80, and 0.083–26.5 μg/mL, respectively. Further dilution with the lowest concentrations in the calibration curves were carried out to afford a series of standard solutions for evaluating the limits of detection (LOD) and the limits of quantity (LOQ) of the compounds. The stock and working solutions were stored at 4 °C.

### 2.3. Preparation of samples and negative control (NC) samples

The contents of QHHZ capsule and powders of XEQR tablet (0.20 g) were weighed precisely and extracted with 20 mL of 60% aqueous ethanol for 30 min in an ultrasonic bath. The extracted solution was centrifuged at 4000 rpm for 10 min. The supernatant was collected and filtered through a 0.45-μm filter, and the filtrate was analyzed directly by HPLC.

**Table 1**
The QHHZ capsule and XEQR tablet products investigated in this work.

| Sample No. | Sample name | Batch No. |
|---|---|---|
| A1 | QHHZ capsule[a] | 070301 |
| A2 | QHHZ capsule | 070904 |
| A3 | QHHZ capsule | 070905 |
| A4 | QHHZ capsule | 070906 |
| A5 | QHHZ capsule | 071201 |
| A6 | QHHZ capsule | 071202 |
| A7 | QHHZ capsule | 080101 |
| A8 | QHHZ capsule | 080302 |
| B1 | QHHZ capsule[b] | 0707006 |
| B2 | QHHZ capsule | 0712010 |
| B3 | QHHZ capsule | 0803001 |
| C1 | QHHZ capsule[c] | 12547107 |
| C2 | QHHZ capsule | 20071102 |
| C3 | QHHZ capsule | 20071203 |
| D1 | QHHZ capsule[d] | 20080101 |
| D2 | QHHZ capsule | 20080102 |
| D3 | QHHZ capsule | 20070403 |
| D4 | QHHZ capsule | 20070905 |
| E | QHHZ capsule[e] | 20070502 |
| F1 | QHHZ capsule[f] | 20080101 |
| F2 | QHHZ capsule | 20080102 |
| F3 | QHHZ capsule | 20080603 |
| G1 | XEQR tablet[g] | 030601 |
| G2 | XEQR tablet | 070901 |
| H | XEQR tablet[h] | 20080401 |

[a] WORLDBEST Pharmaceutical Co. Ltd. (Shanghai, China).
[b] WIN HEART Pharmaceutical Co. Ltd. (Tangshan, Hebei, China).
[c] BODE Pharmaceutical Co. Ltd. (Shenyang, Liaoning, China).
[d] HANFANG Pharmaceutical Co. Ltd. (Dalian, Liaoning, China).
[e] KEDA Pharmaceutical Co. Ltd. (Shenyang, Liaoning, China).
[f] MERRO Pharmaceutical Co. Ltd. (Dalian, Liaoning, China).
[g] LUDE Pharmaceutical Co. Ltd. (Anyang, Henan, China).
[h] YIMINTANG Pharmaceutical Co. Ltd. (Siping, Jilin, China).

The NC samples of QHHZ capsule and XEQR tablet were prepared by deriving one or two herbs from the prescriptions. The herbs were ground into powders with a particle size of 40–60 mesh before use, and NC samples (Table 2) were evenly mixed and prepared the same as the sample preparation protocol.

### 2.4. Apparatus and chromatography analysis

Analyses were done on an Agilent Series 1200 HPLC system consisting of a quaternary delivery system, an auto-sampler and a diode array detector. The chromatographic analysis was carried out on an Agilent Eclipse Plus $C_{18}$ (150 mm × 4.6 mm, i.d., 5 μm) column. The mobile phase comprised methanol (A) 3% phosphoric acid (B)

**Table 2**
The information and preparation protocols of the NC samples.

| NC samples | Method of preparation[a] |
|---|---|
| QHHZ capsule without *C. chinensis* Franch | *S. baicalensis* Georgi:the extract (*G. jasminoides* Ellis:*Radix et Rhizoma Rhei*:*C. aromatica* Salisb = 1.5:1:1, w/w):*Broneo*:*Artificial bezoar* = 0.74:3.59:1:1 (w/w) |
| QHHZ capsule without *S. baicalensis* Georgi | *C. chinensis* Franch:the extract (*G. jasminoides* Ellis:*Radix et Rhizoma Rhei*:*C. aromatica* Salisb = 1.5:1:1, w/w):*Broneo*:*Artificial bezoar* = 10:3.59:1:1 (w/w) |
| XEQR tablet without *C. chinensis* Franch and *P. amurense* Rupr | *S. baicalensis* Georgi:*G. jasminoides* Ellis:*Radix et Rhizoma Rhei*:*Radix gentianae*:*U. macrophylla* Wall:*Realgar*:*Cinnabaris*:*Peppermint oil*:*Juncus effusus* L. = 5:5:2:2:2:2:1:0.2:1 (w/w) |
| XEQR tablet without *S. baicalensis* Georgi | *C. chinensis* Franch:*P. amurense* Rupr:*G. jasminoides* Ellis:*Radix et Rhizoma Rhei*:*R. gentianae*:*U. macrophylla* Wall:*Realgar*:*Cinnabaris*:*Peppermint oil*:*J. effusus* L. = 3:5:5:2:2:2:2:1:0.2:1 (w/w) |

[a] The NC samples were prepared according to the prescriptions of the formula and the production protocol of QHHZ capsule and XEQR tablet.

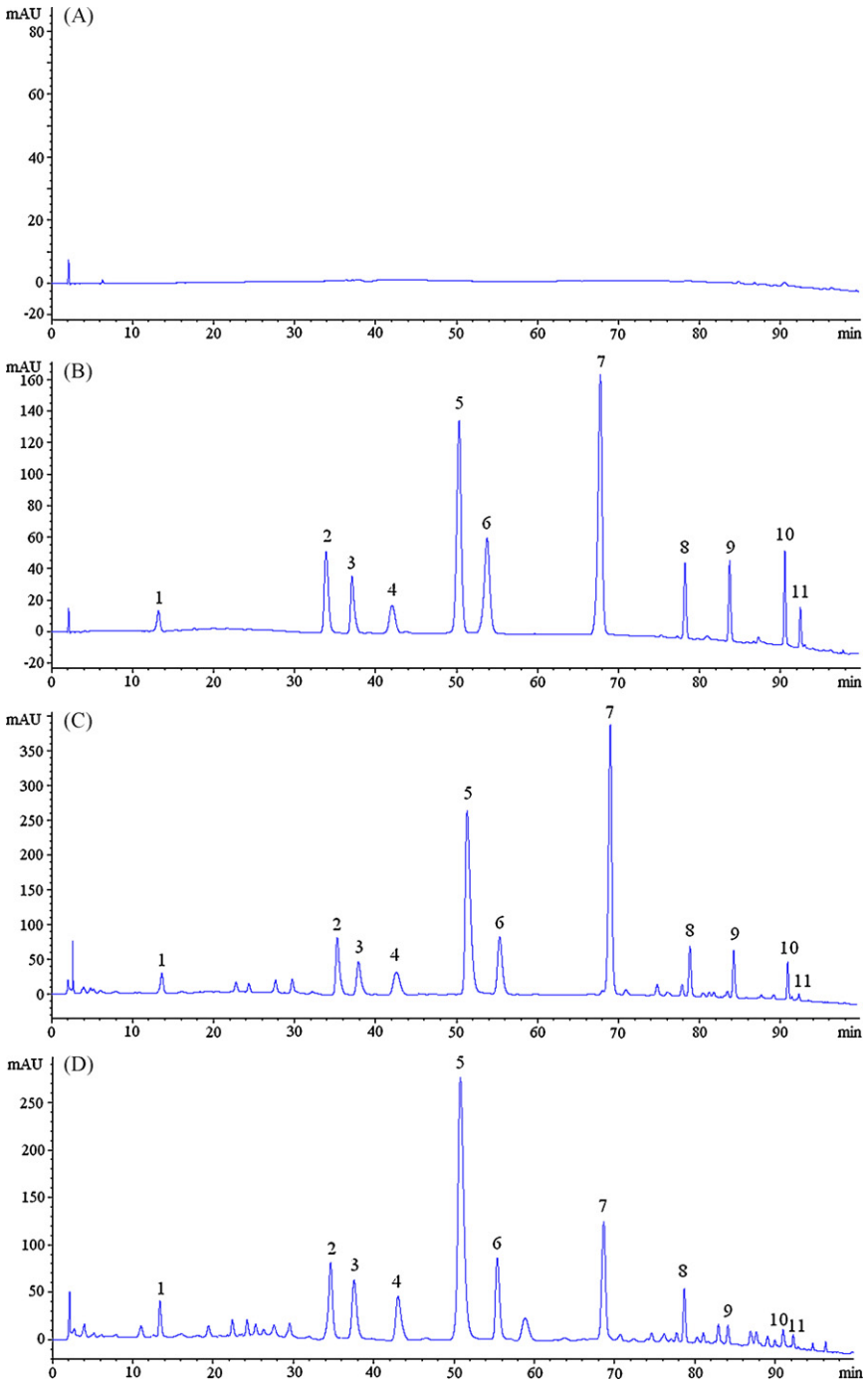


**Fig. 2.** Typical chromatograms for determination of 11 active compounds in QHHZ capsules and XEQR tablets. (A) Sample solvent (60% aqueous ethanol); (B) mixed standards; (C) QHHZ capsule (sample number A6); (D) XEQR tablet (sample number G1). Peak 1 = chlorogenic acid, 2 = coptisine, 3 = epiberberine, 4 = jatrorrhizine, 5 = berberine, 6 = palmatine, 7 = baicalin, 8 = wogonoside, 9 = baicalein, 10 = wogonin and 11 = chrysin, respectively. The separation conditions are described in Section 2.4.

(pH 2.0, adjusted by triethylamine) was used to elute the targets with a gradient mode (0–15 min, 25%A → 30%A; 15–38 min, 30%A; 38–60 min, 30%A → 35%A; 60–85 min, 35%A → 60%A; 85–100 min, 60%A → 90%A). Flow rate and detection wavelength were set at 0.8 mL/min and 270 nm, respectively.

## 3. Results and discussion

### 3.1. Optimization of HPLC conditions

In general, a suitable chromatographic column, mobile phase, elution mode and detection wavelength are critically important for good separation. In the present study, different columns packed with different materials, different mobile phases and elution modes were tested. The columns, i.e., UG120 (250 mm × 4.6 mm, 5 μm), Spherisorb $ODS_2$ (250 mm × 4.6 mm, 5 μm), ZORBAX SB-$C_{18}$ (250 mm × 4.6 mm, 5 μm), Hypersil BDS $C_{18}$ (150 mm × 4.6 mm, 5 μm), Lichrosorb $C_{18}$ (150 mm × 4.6 mm, 5 μm), Eclipse Plus $C_{18}$ (150 mm × 4.6 mm, 5 μm) were employed. Different mobile phases consisting of acetonitrile–water and methanol–water with some modifiers including phosphoric buffer, acetic acid, formic acid, phosphoric acid, and formic acid solutions adjusted by ammonia or triethylamine with different pH values were investigated under different gradient elution modes. The detection wavelength was

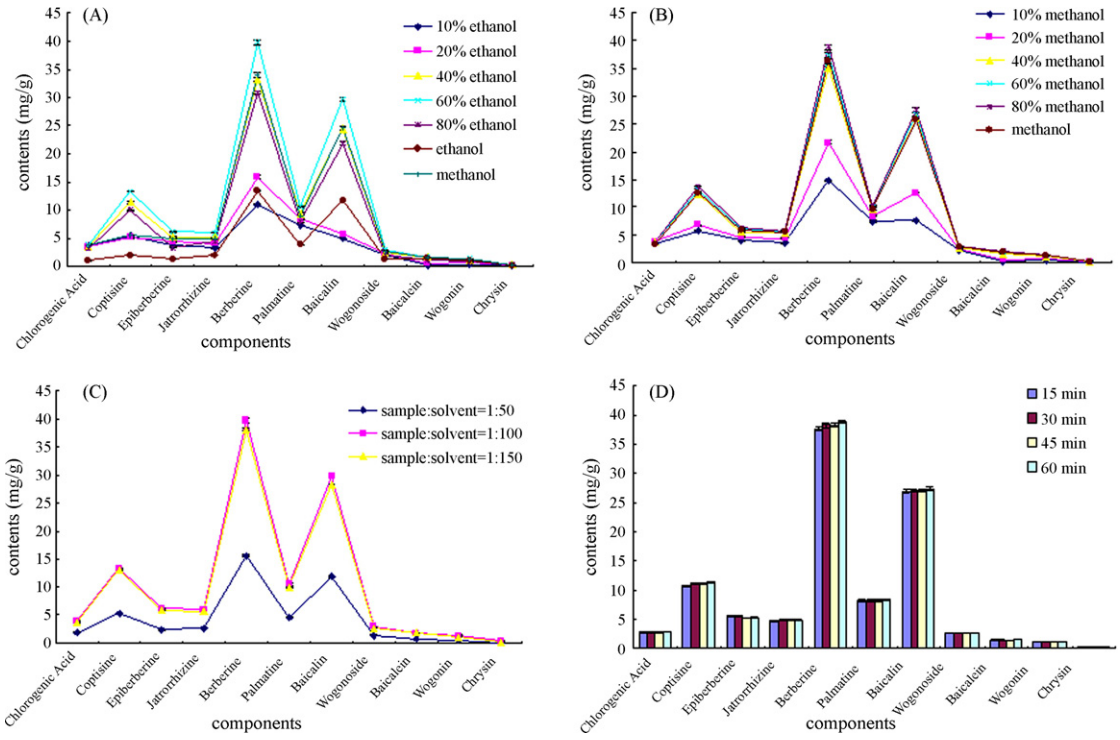


**Fig. 3.** The results of optimization suitable extraction conditions. (A) The influence of different concentrations of ethanol to the compounds (QHHZ capsule sample number A6, sample–solvent ratio = 1:100, w/v); (B) the influence of different concentrations of methanol to the compounds (QHHZ capsule sample number A6, sample–solvent ratio = 1:100, w/v); (C) the influence of sample–solvent ratio to the compounds using 60% ethanol as the extraction solvent (QHHZ capsule sample number A6); (D) the extraction time to the extraction rates of the compounds using 60% ethanol as the extraction solvent (QHHZ capsule sample number A6, sample–solvent = 1:100, w/v).

selected according to the maximum adsorption wavelengths of chlorogenic acid, coptisine, epiberberine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and chrysin at 244, 261, 273, 265, 265, 266, 278, 276, 275, 275 and 268 nm, respectively, shown in UV spectra with three dimension chromatograms of DAD. The flow rate was also optimized. After many tests, excellent separations were achieved and the chromatograms are shown in Fig. 2, in which chromatograms A, B, C and D correspond to blank solvent (60% aqueous ethanol), mixed standards, QHHZ capsule (sample number A6) and XEQR tablet (sample number G1), and the peaks 1–11 represent chlorogenic acid, coptisine, epiberberine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and chrysin, respectively.

### 3.2. Optimization of extraction conditions

Boiling and ultrasonication are often used to extract the targets from the matrix. One disadvantage of boiling is the loss of compounds due to ionization, hydrolysis and oxidation during extraction [27,28]. The other disadvantages are consumption of much solvent, low efficiency of extraction, and time consumption [29,30]. These shortcomings have led to the consideration of ultrasound-assisted extraction (UAE), which has been widely used in TCM analysis [31,32].

In the extraction process, extraction solvent, sample–solvent ratio and extraction time are critical for high extraction efficiency. Pure and aqueous methanol or ethanol solutions are often used as the extraction solvents. In the present study, different concentrations (10%, 20%, 40%, 60%, 80%, and 100%) of ethanol and methanol solutions were examined to extract the targets from QHHZ capsule (sample number A6). The results shown in Fig. 3A and B indicated that the extraction values of all targets gradually increased with increase of the concentrations of ethanol or methanol when the concentration of ethanol was <60% or the concentration of methanol was <80%. High concentrations of ethanol (80% and 100%) or methanol (100%) did not benefit efficient extraction. Sixty percent of aqueous ethanol and 80% aqueous methanol had the highest extraction values (no difference between them). Thus, 60% aqueous ethanol was selected as the extraction solvent. Second, a suitable sample–solvent ratio was investigated and three ratios (1:50, 1:100, and 1:150, w/v) were tested. The sample–solvent ratio controlled at 1:100 was the best (Fig. 3C). Different extract times (15, 30, 45 and 60 min) were also optimized; the extraction time controlled at 30 min was sufficient (Fig. 3D).

Suitable extraction conditions were optimized as follows: samples were extracted by UAE using 100-time of 60% aqueous ethanol as the extraction solvent, and the process lasted for 30 min. A second extraction of the residue was carried out to demonstrate the efficiency of the extraction protocol. No corresponding peaks were detected and all targets were extracted completely (chromatograms not shown).

### 3.3. Method validation

#### 3.3.1. Specificity

NC samples of QHHZ and XEQR were prepared and analyzed to investigate the specificity of the method (Fig. 4). No interferences for determination of the 11 compounds by comparing the retention times with the standards were noted. Purities of the investigated peaks were confirmed to be pure through DAD purity studies.

#### 3.3.2. Calibration curves, LOD and LOQ

The calibration curves were plotted with a series of concentrations of standard solutions. The regression equations were calculated in the form of $Y = aX + b$, where $X$ and $Y$ are the concentration of the standard solution (μg/mL) and the corresponding peak area, and $a$ and $b$ are the slope and the intercept,

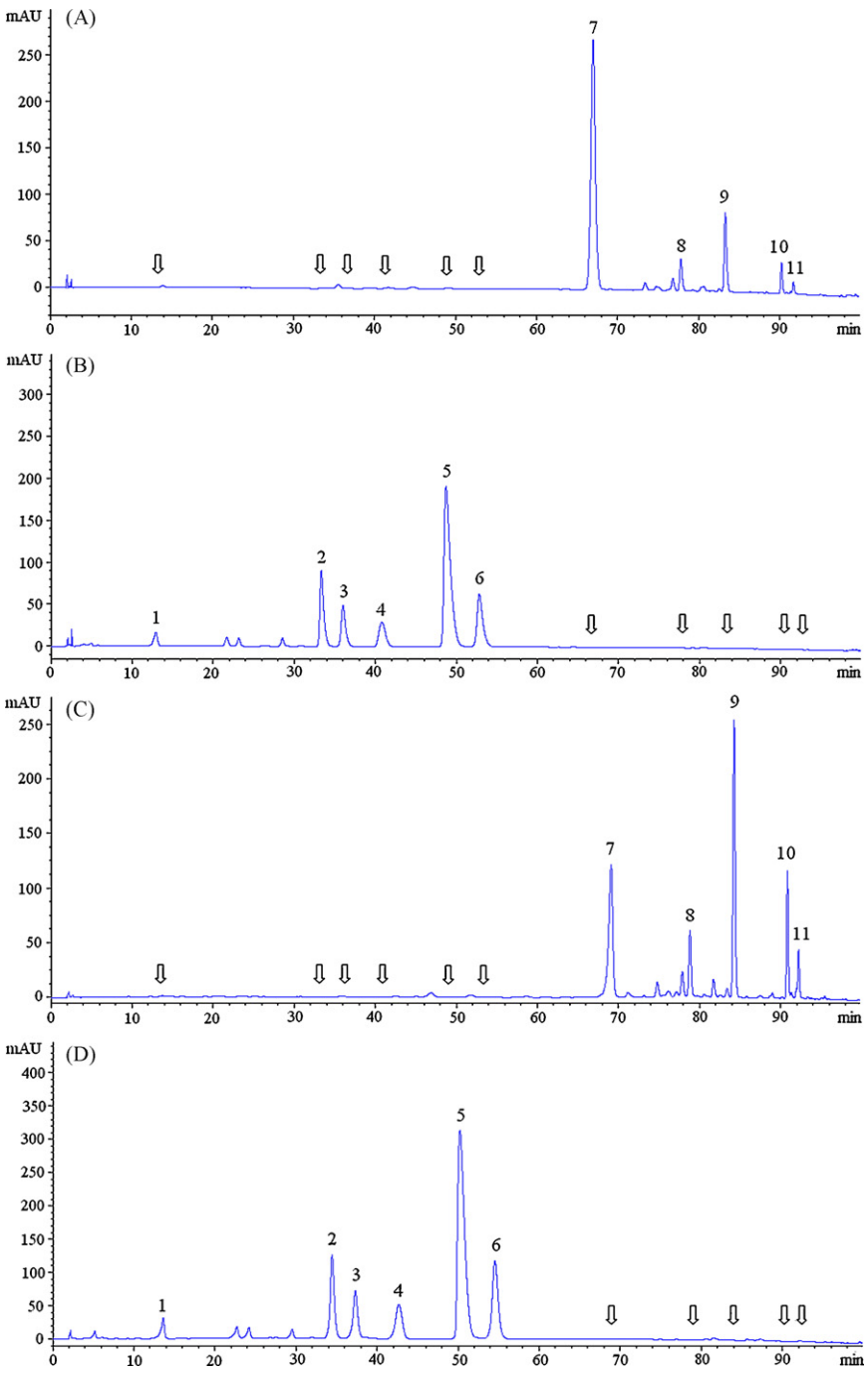


**Fig. 4.** Chromatograms of the NC samples. (A) QHHZ capsule without *R. coptidis*; (B) QHHZ capsule without *R. scutellariae*; (C) XEQR tablet without *R. coptidis* and *C. phellodendri*; (D) XEQR tablet without *R. scutellariae*. The peaks are the same shown in Fig. 2, and the separation conditions are described in Section 2.4.

respectively. Good calibration curves of chlorogenic acid, coptisine, epiberberine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and chrysin were obtained. LOD and LOQ expressed by 3- and 10-fold of the ratio of the signal-to-noise (S/N) were also acquired. Detailed information regarding calibration curves, linear ranges, LOD and LOQ are listed in Table 3.

### 3.3.3. Precision

Instrument precision was evaluated by carrying out intra- and inter-day assays. Intra-day precision was validated with three concentrations of mixed standard solutions under the optimized conditions for five times in 1 day. Inter-day precision was validated with the mixed standard solutions used above for once a day on 5 consecutive days. Inter- and intra-day precisions for all investigated components expressed as relative standard deviation (R.S.D.) were between 0.31% and 2.48% ($n = 5$).

### 3.3.4. Repeatability and stability

Six independent sample solutions of QHHZ capsule (sample number A6) in parallel were prepared and analyzed for evaluation of repeatability. R.S.D. of retention times and peak areas for the 11 compounds were between 0.06% and 0.37%, and 0.96% and 2.03%, respectively. Stability was also tested at room temperature, and samples were analyzed in triplicate every 8 h within 48 h. R.S.D. values were not more than 3.00% for all components.

**Table 3**
Linear relationships between peak area and sample concentration.

| Compound | Regression equation ($Y = aX + b$) | $R^2$ | Linear range (μg/mL) | LOD[a] (μg/mL) | LOQ[b] (μg/mL) |
|---|---|---|---|---|---|
| Chlorogenic acid | $Y = 19.790X - 0.9011$ | 0.9999 | 5.75–184.00 | 0.041 | 0.161 |
| Coptisine | $Y = 30.924X + 66.413$ | 0.9998 | 13.50–270.00 | 0.143 | 0.345 |
| Epiberberine | $Y = 35.112X + 38.758$ | 0.9999 | 15.00–240.00 | 0.164 | 0.374 |
| Jatrorrhizine | $Y = 36.237X + 86.459$ | 0.9995 | 12.75–204.00 | 0.158 | 0.396 |
| Berberine | $Y = 32.187X - 150.96$ | 0.9999 | 100.00–880.00 | 0.104 | 0.245 |
| Palmatine | $Y = 34.589X - 60.54$ | 0.9998 | 12.50–300.00 | 0.113 | 0.359 |
| Baicalin | $Y = 41.713X - 108.39$ | 0.9999 | 50.00–700.00 | 0.108 | 0.301 |
| Wogonoside | $Y = 45.217X + 62.227$ | 0.9999 | 1.625–120.00 | 0.086 | 0.271 |
| Baicalein | $Y = 66.904X + 4.6497$ | 0.9998 | 2.50–80.00 | 0.057 | 0.210 |
| Wogonin | $Y = 63.331X + 55.377$ | 0.9998 | 1.56–50.00 | 0.062 | 0.189 |
| Chrysin | $Y = 42.334X + 2.334$ | 0.9999 | 0.083–26.50 | 0.049 | 0.143 |

[a] LOD refers to the limits of detection, S/N = 3.
[b] LOQ refers to the limits of quantity, S/N = 10.

### 3.3.5. Recovery test

Three quantities (low, medium and high) of the authentic standards were added to the known real sample (QHHZ capsule, sample number A6). Resultants were extracted and analyzed. The quantity of each compound was realized from the corresponding calibration curve. Average recoveries of investigated targets ranged from 97.0% to 100.0%, and R.S.D. values were all <3% ($n = 3$). It was clear that the developed method was reliable and accurate for the measurement.

## 3.4. Sample analysis

The developed method was used to determine the compounds in QHHZ capsules (22 batches) from six manufacturers and XEQR tablets (three batches) from two manufacturers. Contents of the 11 components in the samples are listed in Table 4. Of these, berberine was the main component (>46 mg/g) in QHHZ capsules from MERRO. The second was baicalin (>25 mg/g in all QHHZ capsules), particularly those from WINHEART. The lowest contents of the targets were found in XEQR tablets, and some compounds could not be detected in the sample from YIMINTANG. The alkaloids

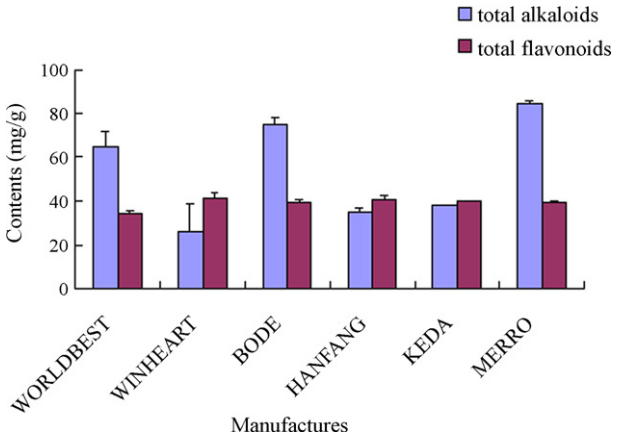


**Fig. 5.** Comparison study of the contents of total alkaloids and total flavonoids in QHHZ capsules collected from different manufactures. Data were expressed as mean ± S.D.

**Table 4**
Determination of the 11 active components in QHHZ capsule and XEQR tablet by the developed HPLC method.

| No. | Contents (mg/g)[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1[b] | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| A1 | 3.53 ± 0.04 | 12.11 ± 0.11 | 7.12 ± 0.14 | 5.37 ± 0.11 | 36.27 ± 0.67 | 9.23 ± 0.14 | 29.75 ± 0.62 | 2.30 ± 0.02 | 1.13 ± 0.02 | 0.51 ± 0.01 | 0.11 ± 0.00 |
| A2 | 3.23 ± 0.05 | 11.62 ± 0.19 | 5.65 ± 0.08 | 4.85 ± 0.10 | 33.64 ± 0.52 | 8.55 ± 0.13 | 29.42 ± 0.58 | 2.96 ± 0.03 | 1.88 ± 0.03 | 1.22 ± 0.02 | 0.22 ± 0.00 |
| A3 | 3.70 ± 0.04 | 10.86 ± 0.17 | 5.17 ± 0.09 | 4.46 ± 0.09 | 31.26 ± 0.47 | 8.51 ± 0.17 | 27.70 ± 0.56 | 2.71 ± 0.01 | 1.92 ± 0.03 | 1.24 ± 0.02 | 0.21 ± 0.00 |
| A4 | 3.16 ± 0.05 | 11.21 ± 0.13 | 5.35 ± 0.11 | 4.79 ± 0.07 | 32.23 ± 0.43 | 8.26 ± 0.11 | 25.98 ± 0.78 | 2.33 ± 0.05 | 1.68 ± 0.04 | 1.05 ± 0.02 | 0.18 ± 0.00 |
| A5 | 3.65 ± 0.03 | 12.99 ± 0.16 | 6.27 ± 0.12 | 5.48 ± 0.10 | 37.37 ± 0.51 | 10.49 ± 0.16 | 27.21 ± 0.46 | 2.83 ± 0.04 | 1.99 ± 0.03 | 1.27 ± 0.02 | 0.23 ± 0.00 |
| A6 | 4.00 ± 0.06 | 13.30 ± 0.19 | 6.17 ± 0.09 | 5.94 ± 0.11 | 39.70 ± 0.62 | 10.53 ± 0.17 | 29.75 ± 0.54 | 2.99 ± 0.04 | 1.64 ± 0.03 | 1.42 ± 0.02 | 0.44 ± 0.01 |
| A7 | 3.34 ± 0.05 | 10.63 ± 0.14 | 4.74 ± 0.07 | 4.42 ± 0.08 | 30.04 ± 0.48 | 8.30 ± 0.16 | 29.02 ± 0.47 | 2.64 ± 0.05 | 1.81 ± 0.02 | 1.08 ± 0.01 | 0.20 ± 0.00 |
| A8 | 3.00 ± 0.04 | 10.98 ± 0.17 | 4.87 ± 0.08 | 4.53 ± 0.10 | 30.16 ± 0.51 | 8.65 ± 0.17 | 28.78 ± 0.56 | 2.82 ± 0.05 | 1.79 ± 0.02 | 1.00 ± 0.01 | 0.21 ± 0.00 |
| B1 | 0.93 ± 0.01 | 2.74 ± 0.04 | 1.67 ± 0.02 | 2.16 ± 0.04 | 8.84 ± 0.14 | 1.97 ± 0.04 | 39.19 ± 0.64 | 1.25 ± 0.01 | 1.17 ± 0.01 | 0.34 ± 0.00 | 0.09 ± 0.00 |
| B2 | 0.97 ± 0.02 | 2.93 ± 0.05 | 1.78 ± 0.03 | 2.49 ± 0.05 | 9.84 ± 0.14 | 2.36 ± 0.05 | 40.28 ± 0.71 | 1.17 ± 0.01 | 1.21 ± 0.01 | 0.35 ± 0.00 | 0.09 ± 0.00 |
| B3 | 2.25 ± 0.04 | 5.36 ± 0.09 | 3.30 ± 0.05 | 3.93 ± 0.09 | 21.76 ± 0.41 | 6.29 ± 0.10 | 36.17 ± 0.62 | 1.12 ± 0.01 | 1.05 ± 0.01 | 0.31 ± 0.00 | 0.10 ± 0.00 |
| C1 | 4.25 ± 0.07 | 10.46 ± 0.17 | 6.44 ± 0.10 | 6.75 ± 0.10 | 42.71 ± 0.62 | 11.89 ± 0.23 | 37.28 ± 0.64 | 2.69 ± 0.02 | 0.57 ± 0.01 | 0.23 ± 0.00 | 0.04 ± 0.00 |
| C2 | 3.84 ± 0.06 | 9.98 ± 0.15 | 6.42 ± 0.11 | 6.03 ± 0.08 | 40.74 ± 0.48 | 10.93 ± 0.17 | 36.24 ± 0.63 | 1.62 ± 0.01 | 0.40 ± 0.00 | 0.27 ± 0.00 | 0.01 ± 0.00 |
| C3 | 4.00 ± 0.05 | 9.99 ± 0.14 | 6.66 ± 0.09 | 6.12 ± 0.09 | 40.64 ± 0.52 | 9.88 ± 0.13 | 36.53 ± 0.70 | 1.80 ± 0.01 | 0.48 ± 0.01 | 0.29 ± 0.00 | 0.01 ± 0.00 |
| D1 | 5.26 ± 0.08 | 5.85 ± 0.12 | 1.97 ± 0.03 | 2.61 ± 0.04 | 18.77 ± 0.21 | 6.53 ± 0.11 | 36.56 ± 0.62 | 2.84 ± 0.05 | 0.67 ± 0.01 | 0.28 ± 0.00 | 0.03 ± 0.00 |
| D2 | 5.41 ± 0.11 | 5.34 ± 0.10 | 2.24 ± 0.04 | 2.63 ± 0.05 | 19.53 ± 0.34 | 6.97 ± 0.19 | 36.90 ± 0.74 | 1.76 ± 0.01 | 1.19 ± 0.01 | 0.41 ± 0.00 | 0.07 ± 0.00 |
| D3 | 5.23 ± 0.10 | 3.86 ± 0.07 | 2.22 ± 0.03 | 2.46 ± 0.03 | 18.82 ± 0.31 | 6.35 ± 0.11 | 37.26 ± 0.84 | 0.43 ± 0.00 | 2.07 ± 0.03 | 0.50 ± 0.00 | 0.13 ± 0.00 |
| D4 | 5.06 ± 0.08 | 5.22 ± 0.07 | 1.90 ± 0.03 | 2.42 ± 0.03 | 18.38 ± 0.32 | 6.36 ± 0.12 | 38.56 ± 0.79 | 2.07 ± 0.04 | 1.60 ± 0.02 | 0.70 ± 0.01 | 0.28 ± 0.00 |
| E | 2.24 ± 0.05 | 6.88 ± 0.09 | 3.84 ± 0.09 | 3.67 ± 0.04 | 19.26 ± 0.35 | 4.62 ± 0.08 | 37.31 ± 0.68 | 1.76 ± 0.02 | 0.96 ± 0.01 | 0.24 ± 0.00 | 0.05 ± 0.00 |
| F1 | 4.68 ± 0.09 | 12.77 ± 0.16 | 6.96 ± 0.12 | 6.76 ± 0.09 | 47.03 ± 0.86 | 12.33 ± 0.24 | 35.58 ± 0.57 | 1.77 ± 0.01 | 0.94 ± 0.01 | 0.43 ± 0.00 | 0.08 ± 0.00 |
| F2 | 5.19 ± 0.07 | 11.47 ± 0.14 | 6.92 ± 0.10 | 6.91 ± 0.11 | 46.64 ± 0.78 | 12.60 ± 0.21 | 37.63 ± 0.71 | 0.67 ± 0.01 | 0.81 ± 0.01 | 0.26 ± 0.00 | 0.09 ± 0.00 |
| F3 | 5.06 ± 0.06 | 11.51 ± 0.16 | 6.79 ± 0.11 | 6.89 ± 0.09 | 46.14 ± 0.81 | 12.37 ± 0.23 | 38.01 ± 0.61 | 0.30 ± 0.00 | 0.99 ± 0.02 | 0.19 ± 0.00 | 0.08 ± 0.00 |
| G1 | 2.93 ± 0.04 | 2.17 ± 0.03 | 1.83 ± 0.02 | 1.76 ± 0.03 | 12.17 ± 0.21 | 4.07 ± 0.07 | 15.45 ± 0.25 | 3.31 ± 0.05 | 2.90 ± 0.05 | 2.12 ± 0.04 | 0.90 ± 0.01 |
| G2 | 3.03 ± 0.05 | 4.17 ± 0.07 | 2.45 ± 0.04 | 2.44 ± 0.04 | 19.36 ± 0.30 | 4.31 ± 0.06 | 5.15 ± 0.08 | 1.05 ± 0.02 | 0.29 ± 0.00 | 0.24 ± 0.00 | 0.27 ± 0.00 |
| H | ND[c] | ND | ND | ND | 0.88 ± 0.01 | ND | ND | ND | 0.98 ± 0.02.0. | 0.85 ± 0.01 | 0.19 ± 0.00 |

[a] Data of the contents are expressed as mean ± S.D. ($n = 3$).
[b] 1 = chlorogenic acid, 2 = coptisine, 3 = epiberberine, 4 = jatrorrhizine, 5 = berberine, 6 = palmatine, 7 = baicalin, 8 = wogonoside, 9 = baicalein, 10 = wogonin and 11 = chrysin.
[c] Not detected.

and flavonoids in *C. chinensis* Franch, *S. baicalensis* Georgi and *P. amurense* Rupr were the main active components. The contents of these components may be considered for quality control of the two medicines. The comparison gram of total alkaloids (TAs) and total flavonoids (TFs) in QHHZ capsule among different manufacturers was made (Fig. 5). This indicated that the highest contents of TAs were detected from MERRO and BODE compared with the drugs from WORLDBEST ($P < 0.05$), WINHEART and HANFANG ($P < 0.01$). The contents of TFs from WINHEART, BODE, HANFANG and MERRO were identical and higher than the samples from WORLDBEST ($P < 0.05$). The contents of TFs and TAs from KEDA were not analyzed statistically because only one sample was collected from this company. The phenomenon described above and the data shown in Table 4 indicated that the contents of the compounds were different in capsules collected from different manufacturers. This may arise from the different resources of *C. chinensis* Franch, *S. baicalensis* Georgi, and the disparity in preparation technologies in different factories. The quality evaluation regarding the QHHZ capsule was that the main 11 compounds could be detected, and the contents of TAs and TFs were >30 and 35 mg/g, respectively. Our HPLC system may be used as a tool to evaluate the quality of natural products.

## 4. Conclusions

Increasing numbers of traditional Chinese herbs are being used worldwide. Efficient protocols to evaluate and control the quality of herbal products are urgently needed. This is the first report for simultaneous determination of the 11 marker compounds in QHHZ capsule and XEQR tablet. The developed method has the advantages of simplicity, precision, accuracy and sensitivity, and is suitable to control the quality of the two medicinal products. This method can be used to control the quality of other related pharmaceutical preparations containing *S. baicalensis* Georgi, *C. chinensis* Franch, or *P. amurense* Rupr. Additional methods must be developed to measure plant species other than *C. chinensis* Franch, *S. baicalensis* Georgi and *P. amurense* Rupr that comprise these pharmaceutical preparations.

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