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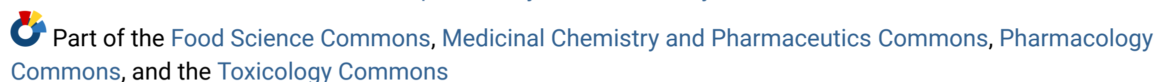
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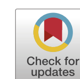
Original Article

# Simultaneous quantification of six indicator compounds in Wen-Qing-Yin by high-performance liquid chromatography-diode array detection

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ABSTRACT

A simple gradient high-performance liquid chromatography with diode array detection (HPLC-DAD) method was used to simultaneously to analyze characteristics of six indicator compounds in the traditional Chinese medicine (TCM) formulation Wen-Qing-Yin (WQY). Separate optimization was performed using a Cosmosil C18 column gradient method with 0.1% formic acid in both mobile phases of aqueous and acetonitrile (ACN), at a flow rate, detection wavelength, and sample volume of 1.8 mL/min, 268 nm, and 10 μL, respectively. The linear regression of six active compounds berberine (BER), baicalin (BAI), ferulic acid (FER), geniposide (GEN), hydorxymethoxylfurfural (HMF), and paeoniflorin (PAE) was produced at the concentration range of 10–2000 μg/mL. The method validation revealed an acceptable precision (intra- and inter-day precision < 3.39% and 4.11%, respectively) and recovery (85.60–110.45% and 86.58–110.90%), a recovery range of 86.61–109.42%, and sensitivity (limit of detection [LOD] and limit of quantification [LOQ] values were in the range of 0.03–3.13, and 0.08–9.38 μg/mL, respectively) while the calibration curves were linear with a correlation coefficient ($R^2$) ranging from 0.9966 to 0.9989. The qualitative and quantitative analyses were performed by direct comparison of the peaks of the WCY extract to retention times of reference standards. Additionally, principal component analysis (PCA) successfully discriminated four purchased commercial samples of all six indicator constituents, and the present results indicate their comprehensive potential usefulness for qualitative and quantitative analyses of the WQY decoction and its commercial products.

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## 1. Introduction

Recently, traditional Chinese medicine (TCM) formulations are gaining increasing attention in numerous fields because of their low toxicity and mild therapeutic activity [1]. In contrast to pharmaceutical drugs that contain specific active constituents and exhibit clear dose-dependent effects and cytotoxic, herbal medicines are often derived from complex systems containing multiple components [2]. Furthermore, all the components in TCM preparations are considered to contribute to mediating the beneficial effects and not just a few active compounds. TCM formulations are composed of diverse components, and their contents vary with growing soil, climate, and the growth stage when harvested [3]. This makes the quality control of crude drugs and their medical preparations extremely difficult; similarly, the quality of medicines is also an important foundation for their clinical efficacy [4]. Traditionally, the contents of active components in TCM formulations have been used to evaluate the quality of raw herbal medicines [5,6].

Wen-Qing-Yin (WQY), a combinatorial formula, includes *Coptidis Rhizoma* (Huang Lian), *Scutellaria Radix* (Huang Qin), *Phellodendri Cortex* (Huang Bai), *Gardeniae Fructus* (Zhi Zi), *Rehmanniae Radix Preparata* (Shu Di Huang), *Chuanxiong Rhizoma* (Chuan Xiong), *Angelicae Sinensis* Radix (Dang Gui), and *Paeoniae Alba* Radix (Bai Shao) in equal proportions. Generally, WQY has been clinically used to regularize the menstrual cycle, clear heat and purge pathogenic fire, resolve toxins, nourish and quicken the blood, and stop bleeding [7]. WQY is composed of Huang Lian Jie Du Tang (HLJDT) and Si Wu Tang (SWT), which has been investigated for hypotensive effects on the theophylline-induced increase in arterial blood pressure [8]. In addition, several studies revealed the exceptional pharmacological activities such as anticancer [9–12], anti-inflammatory [13–17], anti-oxidation [17–20] and antimicrobial [21–24].

Several analytical methods have been reported for the simultaneous determination of indicator compounds in herbal medicines formula, such as ultra-high-performance liquid chromatography (UHPLC) [25], capillary electrophoresis (CE) [26], and HPLC coupled with other detectors including ultraviolet (UV), mass spectrometry [27], a diode array detector (DAD) [28], and electrochemical [29] detection. However, HPLC-DAD is the recommended and most popular technique for the analysis of herbal formula because of its wide choice of mobile phases, column, contamination resistant, and low cost. It plays a crucial role in the compound separation of mix substance to improve the elucidation of the role of individual molecules.

Consequently, it is important to identify suitable methods to monitor the amount of active ingredients in the combinatorial formula in quality control analyses of medicine manufacturers. Currently, very limited studies on WQY have been carried out in the analytical sciences. Hence, a simple and efficient analytical method was developed for the simultaneous detection of the constituents of WQY is important. Especially, principal components analysis (PCA) [30], a mathematical procedure, which is used to minimize data dimensionality combined with HPLC chromatographic data provided on the important information for the evaluation of WQY commercial products. In this study, although we simultaneously quantified six major quality indicators (indicators recorded in Taiwan Herbal Pharmacopoeia for WQY) in WQY by the method established by us, our findings showed that using only a few indicators is not sufficient to determine the quality of commercial products. Moreover, our validated HPLC method was then successfully applied to quantify these crude herbs and commercial products.

## 2. Materials and methods

### 2.1. Chemicals and reagents

HPLC grade methanol (MeOH, Beijing Chemical Reagent Company, Beijing, China) was used for sample extraction, and HPLC grade acetonitrile (ACN) used as the mobile phase were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). Deionized water from a Millipore water purification system (Millipore, Bedford, MA, USA) and analytical grade formic acid (Beijing Chemical Reagent Company, Beijing, China) were used for the HPLC analysis. Berberine (BER), baicalin (BAI), ferulic acid (FER), geniposide (GEN), hydorxymethoxylfurfural (HMF), and paeoniflorin (PAE) with a purity of >95% by HPLC were purchased from Sigma–Aldrich (St. Louis, MO, USA). All standards were filtered using 0.22 μm membranes before use. Four commercial products were purchased from factories of Sun Ten Pharmaceutial Co., Ltd., Chuang Song Zong Pharmaceutial Co., Ltd., Fu Tian Pharmaceutial Co., Ltd., and Sheng Foong Co., Ltd., respectively.

### 2.2. Sample preparations

The raw materials of Coptidis Rhizoma (Huang Lian), *Scutellaria* Radix (Huang Qin), Phellodendri Cortex (Huang Bai), Gardeniae Fructus (Zhi Zi), *Rehmanniae* Radix Preparata (Shu Di Huang), Chuanxiong Rhizoma (Chuan Xiong), *Angelicae sinensis* Radix (Dang Gui), and *Paeoniae alba* Radix (Bai Shao) were powdered using a homogenizer, filtered through a 40-mesh sieve, divided into equal proportions, and dissolved with 100% MeOH in a volumetric flask to a final concentration of 1 mg/mL for decoction extraction. After ultrasonic extraction for 30 min at $28 \pm 1$ °C and 20 kHz frequency, the extract was vacuum-filtered using Whatman No. 1 filter paper and then further filtered through a 0.22 μm membrane. For the determination of BER, BAI, FER, GEN, HMF, and PAE a 10 μL aliquot of the solution was injected into the HPLC-DAD system and analyzed immediately. The contents of the analytes were determined from the corresponding calibration curve.

### 2.3. HPLC apparatus and conditions

The components of the methanolic extract of WQY formula were characterized using HPLC DAD analysis. The HPLC apparatus used was a Waters® Alliance 2695 series chromatograph system (Milford Corp., MA, USA) consisting of a quaternary pump and photodiode array detector, and a reversed-phase column of Cosmosil 5C18-AR-II (4.6 × 150 mm, 5 μm, Nacalai USA, Inc., Kyoto, Japan) was used for the separation. The mobile phase was composed of 0.1% formic acid in water (A) and ACN (B). The gradient conditions were as follows: 0.5 min, 0% B;

10 min, 20% B; 30 min, 30% B; 30 min, 40% B; 20 min, 100% B, and then a 10 min hold before returning to the initial conditions. The flow rate was 1.8 mL/min. The wavelength was set at UV 268 nm, and absorption spectra of the compounds were recorded between 200 nm and 400 nm. The column temperature was 30 ± 2 °C, and the sample injection volume was 10 μL. The compounds were identified by comparing their retention times and UV spectra with those of the standards.

### 2.4. *Method validation for standard analysis*

#### 2.4.1. *Regression equations*

Linear regression analysis of each of the six compounds was performed based on the estimated standard curve in triplicate using six different concentrations. A, b, and rare the coefficients of the regression equation, y = ax + b, x indicates the concentrations of the marker compounds (μg/mL), and y and $R^2$ are the peak area and coefficient of correlation of the equation, respectively. The standard curve was constructed over a concentration range of 10–200 μg/mL for HMF, 200–2000 for GEN and PAE, 20–100 for FER, 600–2000 for BER, and 200–1000 for BAI. The line for each compound was plotted using a linear regression of the peak area vs concentration. The $R^2$ was used to determine the linearity. All the marker compounds showed linearity ($R^2 > 0.995$) in a relatively wide concentration range detected at 268 nm. Furthermore, the concentrations of these compounds in the commercial products were also calculated using the same regression parameters.

#### 2.4.2. *Intra- and inter-day precision and recovery*

To assess the recovery of the data, the proximity of the obtained value to the "true" value can be estimated similarly to recovery. By adding known amounts of three concentration levels (low, medium, and high) of the six marker compounds to the powdered sample, after sample preparation and extraction, the concentration of recovery of the substances can be calculated (standard addition method). The precision results of the developed analytical method were expressed as the relative standard deviation (RSD) of multiple samplings of the same homogeneous sample. Precision and recovery were estimated as intra-day and inter-day.

#### 2.4.3. *System validation, repeatability, and stability*

The intra- and inter-day precision of the developed analytical procedure obtained from multiple samplings of the mixed compound standard (six in a single day and three within 3 days). The repeatability of the developed method was estimated by sampling six times a day. The intra- and inter-day precision was determined from six analyses in the same day and three in 3 days. Furthermore, the stability was analyzed by injecting three aliquots of a sample solution during three time-points, 0, 24, and 48 at 15 °C ± 1. The RSD was considered a measure of the precision.

#### 2.4.4. *Limit of detection (LOD) and limit of quantification (LOQ)*

The limit of detection (LOD) and limit of quantification (LOQ) are considered the lowest compound concentration detectable and the exact value determinable with suitable precision and recovery, respectively. The LOD and LOQ were determined at a signal to noise ratio (S/N) of 3 and 10, respectively as the lowest concentrations of the analyte.

#### 2.4.5. *Recovery*

An appropriate amount of the WQY decoction to be extracted was divided into one portion as the control group, and another three portions that were spiked with marker standards at three concentration levels (low, medium, and high). After all the samples were extracted with MeOH and then filtered through a 0.45 μm membrane filter, the filtrates were assayed using HPLC to determine the recoveries, which were calculated using the following equation: recovery (%) = (total amount detected − amount original)/amount spiked × 100.

#### 2.4.6. *Quantification of marker components*

For the crude herb analysis, 2 g samples of pulverized Coptidis Rhizoma, *Scutellaria baicalensis*, Cortex Phellodendri, Fructus Gardeniae, *Rehmanniae* Radix Preparata, *Chuanxiong* Rhizoma, *A. sinensis* Radix, and *P. alba* Radix were extracted with 20 mL of 99.5% methanol by sonication at 28 ± 1 °C for 30 min. The suspension was centrifuged at 3000 rpm for 10 min, and then filtered using a 0.22 μm filter and analyzed by HPLC.

## 3. Results

### 3.1. *Optimized HPLC condition*

The structures of these compounds are shown in Fig. 1. The absorption maxima of HMF, GEN, PAE, FER, BER, and BAI were in the range of 200–400 nm on the ultraviolet (UV) full-spectra with three-dimensional chromatograms and a monitoring wavelength for quantitative determination at 268 nm was altered to obtain the baseline separation of marker compounds. As the polarity and other properties of the various marker substances differ greatly, gradient elution was carried out to successfully separate the compounds in the WQY decoction using HPLC with 0.1% (v/v) formic acid-ACN as the mobile phase within ~65 min (Fig. 2).

### 3.2. *Linear regression equations, LOD and LOQ*

The calculated results are shown in Table 1. All the marker substances showed linearity ($R^2 > 0.995$) in a relatively wide

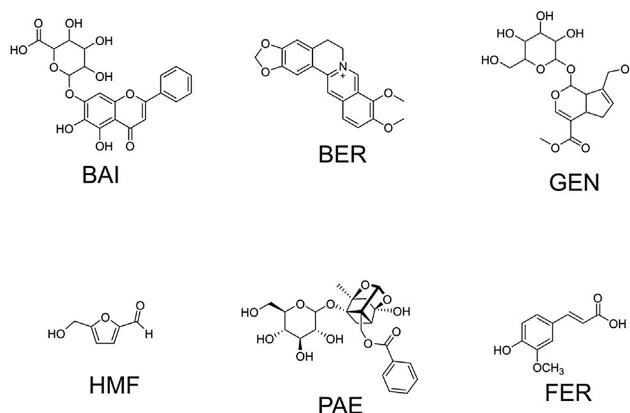


**Fig. 1 – Structures of the six indicator compounds.**

concentration range. The LODs and LOQs were detected at 268 nm.

### 3.3. *Intra- and inter-day precision and recovery*

The within-day and day-to-day precision and recovery data of each marker compound are shown in Table 2a. The RSD of the within-day and day-to-day were <3.39–4.11%, and the recovery ranged from 85.60 to 110.45% and 86.58–110.90%, respectively.

### 3.4. *Precision, repeatability, and stability*

The repeatability was examined using six batches of the sample solutions, and Table 2b shows that the RSD of the six compounds ranged from 0.02 to 0.83%. One of the sample solutions was injected into the HPLC-DAD system at 0, 24 and 48 h, to determine the stability of the solution and it showed RSD values ranging from 0.75 to 1.92%.

### 3.5. *Recovery*

The average recoveries of six standards spiked into the WQY extract at low, medium, and high levels ranged from 101.05 to 109.42% for HMF, 101.70 to 101.29% for GEN, 106.08 to 99.17% for PAE, 87.54 to 86.61% for FER, 95.52 to 94.81% for BER and 101.17 to 92.87% for BAI.

### 3.6. *Determination of six compounds in WQY*

Fig. 2 shows the chromatogram of the HPLC-separated mixture of the six standards and the prepared WQY. The retention times of the six compounds were 2.7, 20.8, 25.5, 27.9, 56.1, and 63.1 min for HMF, GEN, PAE, FER, BER, and BAI, respectively. The qualitative and quantitative data of the analyzed commercial products are shown in Fig. 3 and Table 3.

### 3.7. PCA

The clear separation of different WQY products containing the six indicator compounds was observed in the PCA score plot, where each coordinate represented a sample. The PCA of the HPLC data of four WQY products was divided into four groups (Fig. 4). Samples A, B, C, and D from the factories of Sun Ten Chuang, Song-Zong, Fu Tian, and Sheng Foong were attributed to groups A, B, C, and D, respectively. The difference between these WQY products was attributed to their different manufacturers, plant origins, material harvest time, and extraction processes.

## 4. Discussion

### 4.1. *Optimization of separation conditions*

Proper solvent selection is essential and commonly used to maximize extraction yield recoveries. MeOH was used to extract six compounds from the crude samples because it has very clear advantages, such as the fewest steps, shorter extraction time than other solvents, and good efficacy. However, this extraction solvent was also recommended for the six indicator compounds in the second edition of the Taiwan Herbal Pharmacopeia.

The maximum absorption wavelengths of HMF, GEN, PAE, FER, BER, and BAI were at 285, 238, 230, 320, 345, and 254 nm, respectively; however, all components were acquired in the UV full-spectral wavelength ranging from 200 to 400 nm and a wavelength of 268 nm was the optimized setting for

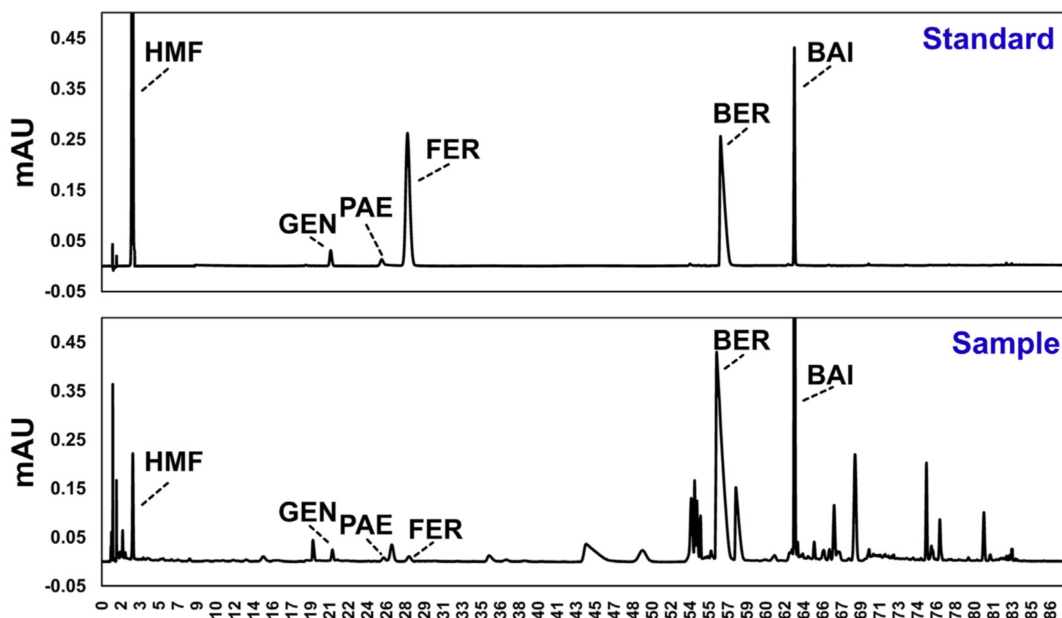


**Fig. 2 – (A) High-performance liquid chromatography (HPLC) peaks of six indicator compounds. HMF, hydroxymethyl furfural; GEN, geniposide; PAE, paeoniflorin; FER, ferulic acid; BER, berberine; BAI, baicalin. (B) Six indicator compounds in self-prepared Wen-Qing-Yin (WQY).**

**Table 1 – Calibration parameters of high-performance liquid chromatography-diode array detection (HPLC-DAD) analysis of six compounds.**

| Compound[a] | Retention time | Regression equation[d] | $R^2$ | Standard curve (μg/mL) | LOD[b] (μg/mL) | LOQ[c] (μg/mL) |
|---|---|---|---|---|---|---|
| HMF | 2.7 | $y = 5881x - 18999$ | 0.9967 | 10–200 | 0.15 | 0.46 |
| GEN | 20.8 | $y = 555x - 11368$ | 0.9972 | 200–2000 | 3.13 | 9.38 |
| PAE | 25.5 | $y = 313x - 12227$ | 0.9981 | 200–2000 | 3.13 | 9.38 |
| FER | 27.9 | $y = 9513x + 5542$ | 0.9966 | 20–100 | 0.16 | 0.46 |
| BER | 56.1 | $y = 24063x - 1521369$ | 0.9989 | 600–2000 | 0.03 | 0.08 |
| BAI | 63.1 | $y = 19983x - 684951$ | 0.9976 | 200–1000 | 0.03 | 0.08 |

[a] Hydroxymethyl furfural, HMF; geniposide, GEN; paeoniflorin, PAE; ferulic acid, FER; berberine, BER; baicalin, BAI.
[b] LOD = 3 × signal-to-noise ratio.
[c] LOQ = 10 × signal-to-noise ratio.
[d] In the regression equation $y = ax + b$, x and y indicate the concentration (μg/mL) and peak area (mAU), respectively. $R^2$ is the correlation coefficient of the equation.

**Table 2a – Intra- and inter-day precision and recovery.**

| Compound[a] | Theoretical conc. (μg/mL) | Found conc. (μg/mL) | | Precision (%) | | Recovery (%) | |
|---|---|---|---|---|---|---|---|
| | | Intra-day[b] | Inter-day[c] | Intra-day[b] | Inter-day[c] | Intra-day[b] | Inter-day[c] |
| HMF | 20 | 20.93 | 20.28 | 3.39 | 3.03 | 104.63 | 89.19 |
| | 40 | 39.43 | 41.09 | 1.60 | 4.11 | 98.56 | 90.34 |
| | 60 | 66.27 | 66.54 | 1.55 | 1.57 | 110.45 | 110.90 |
| GEN | 200 | 201.31 | 198.61 | 1.24 | 1.22 | 99.30 | 100.66 |
| | 400 | 367.77 | 375.86 | 0.83 | 1.96 | 93.96 | 91.94 |
| | 600 | 626.98 | 620.72 | 0.11 | 0.91 | 103.45 | 104.50 |
| PAE | 200 | 198.13 | 205.37 | 0.43 | 3.80 | 99.06 | 102.68 |
| | 400 | 379.37 | 391.95 | 2.13 | 3.08 | 94.84 | 97.99 |
| | 600 | 572.74 | 582.20 | 0.57 | 1.98 | 95.46 | 97.03 |
| FER | 20 | 16.92 | 17.32 | 1.32 | 1.98 | 84.60 | 86.58 |
| | 40 | 36.16 | 36.30 | 0.80 | 0.89 | 90.41 | 90.75 |
| | 60 | 52.38 | 52.91 | 2.25 | 2.44 | 87.30 | 88.18 |
| BER | 600 | 566.11 | 582.31 | 0.58 | 3.83 | 94.35 | 97.05 |
| | 800 | 776.18 | 772.57 | 0.45 | 1.66 | 97.02 | 96.57 |
| | 1000 | 950.99 | 942.41 | 0.17 | 1.32 | 95.10 | 94.24 |
| BAI | 200 | 198.79 | 201.71 | 1.18 | 1.32 | 99.39 | 100.86 |
| | 400 | 343.99 | 348.51 | 0.33 | 1.80 | 86.01 | 87.13 |
| | 600 | 524.76 | 548.91 | 1.65 | 3.87 | 87.46 | 91.48 |

[a] Hydroxymethyl furfural, HMF; geniposide, GEN; paeoniflorin, PAE; ferulic acid, FER; berberine, BER; baicalin, BAI.
[b] Intra-day at three times in 1 day.
[c] Inter-day on 3 different days.

**Table 2b – Precision analysis of standard and stability and repeatability of Wen-Qing-Yin (WQY) samples.**

| Compound[a] | Precision RSD (%) | | Repeatability RSD (%) (six batch per day) | [d]Stability RSD (%) ([c]within 3 days) |
|---|---|---|---|---|
| | Intra-day[b] | Inter-day[c] | | |
| HMF | 0.96 | 0.90 | 0.66 | 2.92 |
| GEN | 0.32 | 0.23 | 0.83 | 1.68 |
| PAE | 0.33 | 0.24 | 0.24 | 0.75 |
| FER | 0.41 | 0.33 | 0.25 | 1.29 |
| BER | 0.05 | 0.05 | 0.10 | 1.26 |
| BAI | 0.01 | 0.02 | 0.02 | 1.34 |

[a] Hydroxymethyl furfural, HMF; geniposide, GEN; paeoniflorin, PAE; ferulic acid, FER; berberine, BER; baicalin, BAI.
[b] Intra-day at three times in 1 day.
[c] On 3 different days.
[d] Stored at 15 °C.

simultaneous quantitative determination to obtain the baseline separation of the marker compounds.

As shown in Fig. 2 (B), the chromatograms of the six marker compounds in the WQY were successfully achieved with a good resolution and symmetric peaks. The peak identification results of six marker compounds were confirmed against retention time of each standards using the same elution program for the HPLC analysis.

Although the polarity and other characteristic factors of each marker compounds were significantly strong and caused long elution times (~80 min), the gradient elution successfully separated the compounds in the WQY extract using 0.1% (v/v) formic acid-ACN as the mobile phase. No interfering peaks were detected in the time frame of any of the six monitored compounds in the WQY decoction. Fig. 2 (B) shows that HMF, GEN, and PAE were at the high polar region during 0–52 min (below ~17% of ACN) and the composition of the solvent system was slowly changed to acquire good separation capacity. Although all the components could be obtained at a good

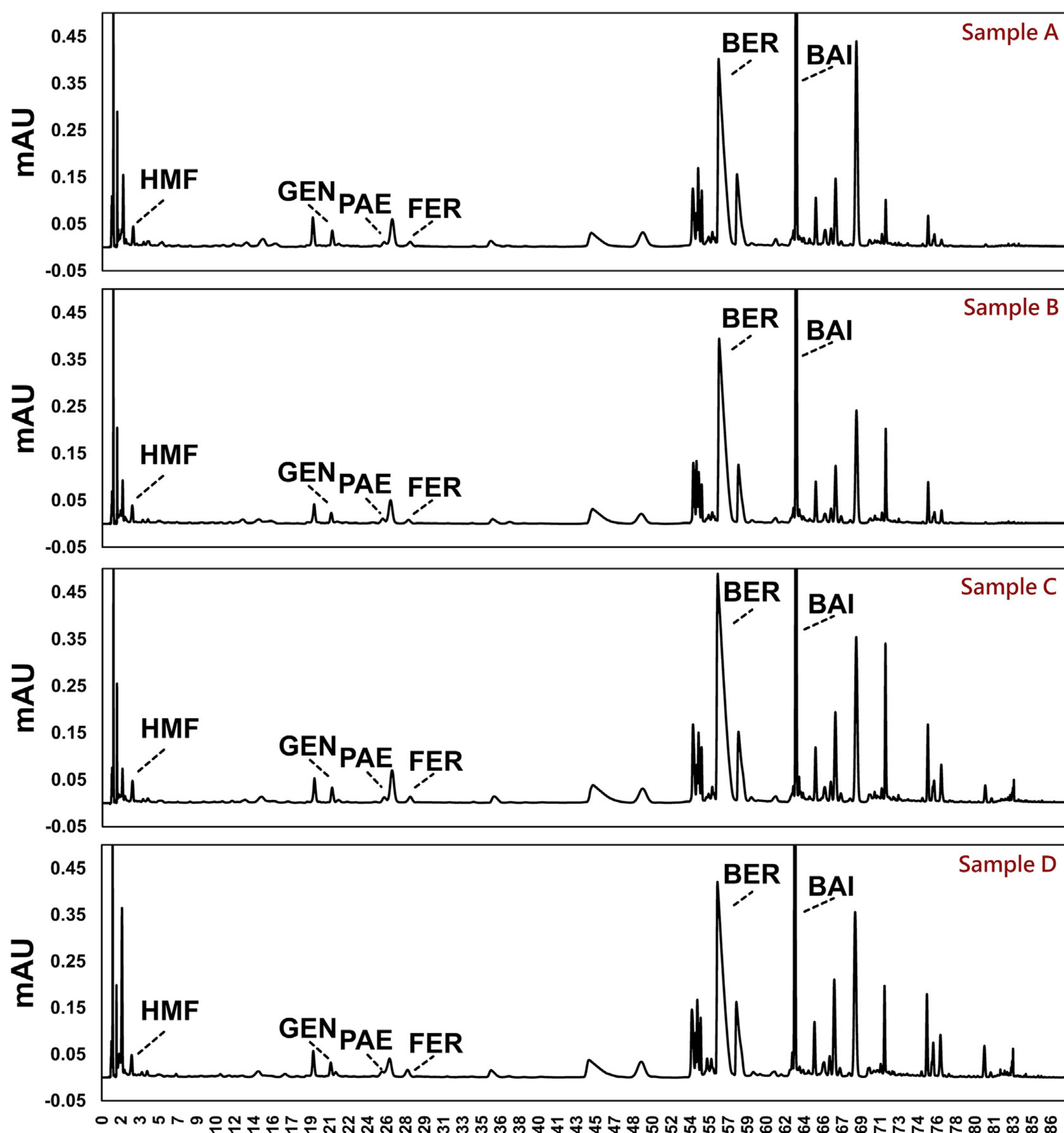


**Fig. 3 – Principle component analysis (PCA) of four commercial products.**

resolution using a higher speed flow rate (1.8 mL/min), the analytical time would be impaired by a prolonged elution.

### 4.2. *Linear curve*

Linear regression analysis of each of the six compounds was established using the external standard method to be evaluated using the $R^2$, range, LOD, and LOQ, which are presented in Table 1. All the marker compounds showed linearity with $R^2 >$ 0.995 at the test concentration range. The LOD and LOQ range at a signal to noise ratio of 3 and 10 for the analytes were 0.03–3.13 and 0.08–9.38 μg/mL, respectively.

### 4.3. *Precision and recovery*

The precision and recovery of the method for each analyte expressed as the RSD and percentage (recovery) were calculated for each standard concentration as required for assay

**Table 3 – Content of six compounds in four different commercial products.**

| Product | Contents[a] [μg/mL, n = 3 (RSD)] | | | | | |
|---|---|---|---|---|---|---|
| | HMF | GEN | PAE | FER | BER | BAI |
| Sample A | 52.98 (0.41) | 507.32 (0.49) | 504.48 (4.09) | 18.63 (0.94) | 647.87 (0.27) | 613.44 (0.22) |
| Sample B | 53.87 (0.42) | 747.26 (0.52) | 378.76 (0.41) | 23.23 (0.16) | 637.87 (0.09) | 617.81 (0.28) |
| Sample C | 63.88 (0.95) | 651.66 (0.16) | 264.96 (0.56) | 31.54 (0.97) | 691.70 (0.13) | 567.85 (0.03) |
| Sample D | 65.33 (0.29) | 769.39 (0.40) | 474.35 (2.39) | 29.35 (3.33) | 897.46 (0.32) | 545.36 (1.04) |

[a] Hydroxymethyl furfural, HMF; geniposide, GEN; paeoniflorin, PAE; ferulic acid, FER; berberine, BER; baicalin, BAI. RSD, relative standard deviation.

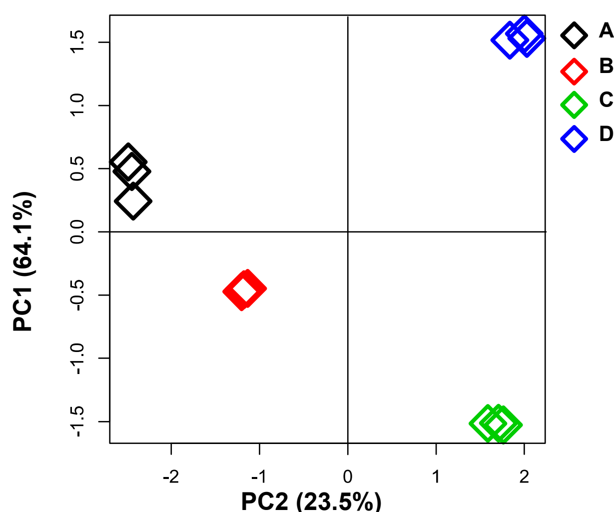


**Fig. 4 – High-performance liquid chromatography (HPLC) peaks of the six indicator compounds in four commercial products. HMF, hydroxymethyl furfural; GEN, geniposide; PAE, paeoniflorin; FER, ferulic acid; BER, berberine; BAI, baicalin.**

validation. Table 2a shows the intra- and inter-day precision and recovery that were tested by spiking samples with three concentration levels (low, medium, and high concentration) of each marker compounds.

The variation of intra-day RSD values ranged from 0.43 to 3.39%, 0.33–2.13%, and 0.11–2.25% at the low, medium, and high levels, respectively and the corresponding inter-day RSD values ranged from 1.22 to 3.83%, 0.89–4.11%, and 0.91–3.87%, respectively. Satisfactory values of the intra-day recovery ranged from 85.60 to 104.63%, 86.01–98.56%, and 87.30–110.45% at low, medium, and high levels, respectively while the corresponding inter-day values ranged from 86.58 to 102.68%, 87.13–97.99%, and 88.18–110.90%, respectively.

Intra-day and inter-day precisions were carried out using six replicate injections of a mixed standard solution during a single day and within 3 consecutive days, and the repeatability was investigated using six batches of the working solutions and one portion was injected into the HPLC at time intervals of 0, 24 and 48 h at 15 °C for determining the stability of the solution. The data are shown in Table 2b, and RSD values of the intra- and inter-day, repeatability, and stability of the six compounds were all <2.92%.

Three levels of each of the six compounds were added to the sample to test the recovery.

The recovery data was between 86.61% and 109.42% with RSD values < 2.74% for all six compounds. Therefore, this established method showed reliability under optimized conditions.

### 4.4. *Application for determination of six marker compounds in commercial WQY products*

The developed HPLC method was validated and used to determine the components in the herbal extract and commercial WQY products. The tentatively identified peaks of the marker compounds in the chromatograms were characterized based on their retention, which was confirmed by comparison with the pure individual standard (Fig. 2). To obtain satisfactory extraction efficiency, MeOH was chosen as the extraction solvent because of its simplicity, low toxicity, and low cost (commercial application). In commercial products of WQY, the six indicator compounds could be determined individually in the chromatogram of WQY. The composition of the TCM pharmaceutical product is quite complex, industrial dependent, and some components may be altered during the manufacturing process. Hence, the contents of the analytes in the commercial products differed from those of the individual single herbs shown in Table 3. According to our result, the remarkable difference in the contents of the six compounds between the various commercial products was identified in the chromatogram as well as the PCA analysis. This variability in content might have been caused by the differences in geographic source, cultivating conditions, or manufacturing process.

## 5. Conclusion

Although HPLC analytical methods are currently used to determine active compounds in many natural resources, there is a need to develop more robust, simultaneous quantification methods for analyzing phytochemicals of interest. The HPLC-DAD method developed in this study was shown to be sensitive, accurate, and suitable for routine analysis of commercial WQY products. These results suggest that the established method was validated to exhibit satisfactory recovery, reproducibility, and precision for simultaneous analysis. To the best of our knowledge, this is the first report of simultaneous determination of the six major compounds in WQY decoction. With a relatively simple extraction procedure that is cost saving, the developed HPLC-DAD method combined with PCA based on the validation results of good recovery and precision could be efficiently used to qualitatively and quantitatively assess the contents of the indicator marker compounds in different WQY commercial products. Although this study used PCA, a reliable method for evaluating the quality of WQY products, the analyte contents in the commercial products in our study were different from those of the individual single herbs. Taken together, our findings indicate that the developed HPLC-DAD method rapidly and efficiently analyzed the constituents of WQY preparations, but the utilization of PCA as a quality control tool for commercial products should be further improved by monitoring more indicator marker compounds of individual medicinal herbs.

## Declarations of interest

None.

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## Data statement

All data generated or analyzed during this study are included in this published article.


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