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Contents of major bioactive flavones in proprietary traditional Chinese medicine products and reference herb of *Radix Scutellariae*

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ABSTRACT

A simple and efficient HPLC/UV method for the simultaneous determination of six bioactive flavones, namely baicalein, baicalin, wogonin, wogonoside, oroxylin A and oroxylin A-7-*O*-glucuronide, has been developed and applied for their content determination in reference herb and proprietary traditional Chinese medicine (PTCM) products of *Radix Scutellariae*. The chromatographic separation was carried out on a Thermo C_{18} column and linear gradient elution was employed with a mobile phase containing acetonitrile and 20 mM sodium dihydrogen phosphate buffer (pH 4.6). All the analytes were detected by PDA detector at a wavelength of 270 nm. Contents of the analytes in *Radix Scutellariae* containing PTCM products in forms of capsule, soft capsule, tablet and dripping pill and the reference herb of *Radix Scutellariae* were analyzed by sonicator extraction with methanol and water mixture (80:20) containing 1 mM HCl for 30 min followed by HPLC analysis. Separation of the six analytes was achieved within 25 min with good linearity ($R^2 > 0.99$). The R.S.D. of both the intra-day and inter-day precision for all the six analytes was below 10.14%. The accuracy at different concentrations was within the range of −7.83 to 4.06%. The extraction recovery was within the range of 89.22–107.33% for all the analytes. Contents of the six flavones were found to vary significantly among different products with glycosides, such as baicalin, wogonoside and oroxylin A-7-*O*-glucuronide, in much greater quantity than their corresponding aglycones. In addition to baicalin (18.54 ± 0.71%, w/w), the commonly used marker compound for *Radix Scutellariae*, wogonoside (3.54 ± 0.18%, w/w) and oroxylin A-7-*O*-glucuronide (2.84 ± 0.14%, w/w) also existed in abundant amount in the reference herb. Our findings suggested that wogonoside and oroxylin A-7-*O*-glucuronide should also be served as the chemical markers together with baicalin for the quality control of herbs and PTCM products of *Radix Scutellariae*.

1. Introduction

Scutellaria baicalensis Georgi is a medicinal plant widely distributed in Asia. Its dried root, *Radix Scutellariae* (Huangqin), is officially listed in the Chinese Pharmacopeia and recognized as bioactive ingredients in a number of proprietary traditional Chinese medicines mainly to treat inflammation, cardiovascular diseases, respiratory and gastrointestinal infections. Water extract of *Radix Scutellariae* had been reported to have *in vitro* anti-inflammation effect and anticonvulsant effect via the prevention of seizure spread in mice [1–3]. Methanol extract of *Radix Scutellariae* has been found to have strong anti-oxidative effect and be able to inhibit the proliferation of human monocytic leukemia cell line THP-1 and human osteogenic sarcoma cell line HOS [4,5].

Flavonoids are the most abundant ingredients in *Radix Scutellariae* with over 30 of them have been identified and quantified by various analytical techniques [6]. Six flavones, namely baicalein (B), baicalin (baicalein-7-glucuronide, BG), wogonin (W), wogonoside (wogonin-7-glucuronide, WG), oroxylin A (OA) and oroxylin A-7-glucuronide (OAG) were reported to be the major bioactive components in *Radix Scutellariae* [7]. The chemical structures of the six major flavones are shown in Fig. 1. Other identified flavonoids in *Radix Scutellariae* include 5,2′-dihydroxy-6,7,8,3′-tetramethoxyflavone, skullcapflavone I, chrysin 8-C-β-D-glucopyranoside, viscidulin III-2′-O-β-D-glucoside, 5,7,2′,5′-tetrahydroxyflavone, (−)-eriodictyol and rivularin [8–10].

Similar to the *Radix Scutellariae* extract, the pure components of B, W, OA, BG, WG and OAG have also demonstrated various pharmacological effects, among which the anti-inflammation effect has been extensively studied. B, W, OA and BG have all showed their anti-inflammatory effect mainly by inhibiting the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) gene expression [11–16]. In addition, B, BG, W and WG have demonstrated anti-oxidative effect [17,18]. Furthermore, anticancer effects of the major flavonoid components in *Radix Scutellariae* such as

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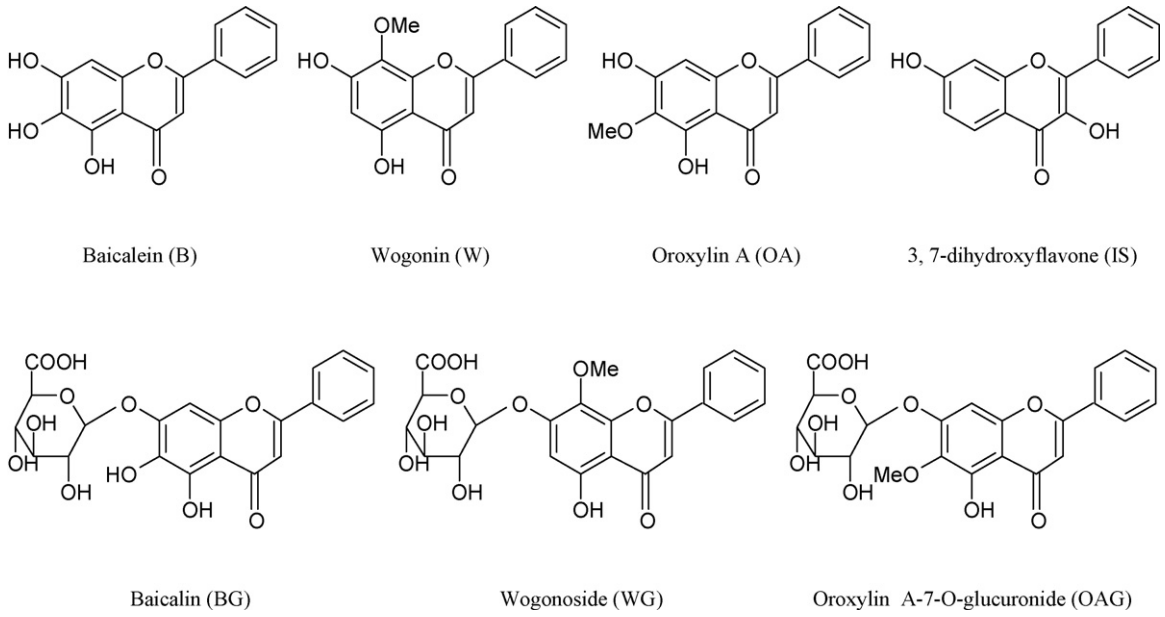

Fig. 1. Chemical structures of major bioactive flavones in *Radix Scutellariae* and 3,7-dihydroxyflavone (internal standard).

B, BG, W and OA have been confirmed in a number of *in vivo* studies and cell models including human/murine bladder cancer cells, promyeloleukemic cells and human hepatocellular carcinoma HepG2 cells [19–23]. The aglycones including B, W and OA also exhibited anti-viral effects [24–28]. Recently, there are quite a few reports on the effect of *Radix Scutellariae* components on central nervous system. OA and B have showed neuroprotective effect in memory impaired mice [29–31]. The anxiolytic effect of B and W was also observed in mice [32,33].

Due to their effectiveness and relatively low toxicity, herbal medicines have drawn more and more attention during the past decades. With the growing use of herbal products, its quality becomes especially important to guarantee the safety and efficacy of the utilization of herbal medicines. BG is identified as the marker compound for quality control of *Radix Scutellariae* and its content is requested to be not less than 9% (w/w) in dried *Radix Scutellariae* in Pharmacopeia of PRC 2005 [34]. There are a number of studies concerning the content determination of the bioactive components in *Radix Scutellariae* using various assay methods, in which chromatography methods are frequently employed. These detection methods include high-performance liquid chromatography [35–39], capillary electrophoresis [40], microemulsion electrokinetic chromatography [41] and micellar electrokinetic capillary electrophoresis [7]. Most studies just chose baicalin and/or baicalein as the marker compounds for quality control. However, there are evidences showing that the other components, including WG, OAG, W and OA, also exist in relatively high amount. It has been reported that a herb couple (*Coptidis Rhizoma*:*Radix Scutellariae*, 1:1) was extracted by 70% methanol and the contents of BG, WG, OAG, B, W and OA were determined to be 84.82 ± 3.02, 78.69 ± 0.69, 40.57 ± 0.65, 18.28 ± 0.06, 9.32 ± 0.19, and 4.01 ± 0.07 mg/g (w/w), respectively by micellar electrokinetic capillary electrophoresis [7]. In another study, *Radix Scutellariae* was extracted by high-speed counter-current chromatography and the contents of B, W and OA were 3.58, 1.23 and 0.31% (w/w), respectively using HPLC/UV analysis [42]. Unlike the synthetic drugs, herbal medicines have more complicated compositions. The effectiveness of herbal medicines may be attributed to the overall effect of all the components rather than a single component. Besides, the interactions among different components in different herbs are always a concern. Thus, the quality evaluation of herbal medicine should contain the information of as much bioactive components as possible.

The present study is proposed aiming to develop a simple HPLC/UV method for the simultaneous determination of six bioactive flavones (baicalein, baicalin, wogonin, wogonoside, oroxylin A and oroxylin A-7-*O*-glucuronide) in the reference herb and commercial PTCM of *Radix Scutellariae*, which could provide a more suitable method and significantly improve the quality evaluation of the raw material and commercial PTCM of *Radix Scutellariae*.

2. Experimental

2.1. Chemicals and reagents

Baicalein (B) and baicalin (BG) with purity over 98% were purchased from Sigma–Aldrich Chem. Co. (Milwaukee, WI, USA). Wogonin (W) and wogonoside (WG) with purity over 98% were purchased from AvaChem Scientific LLC (San Antonio, TX, USA). Oroxylin A (OA, purity over 98%) and oroxylin A-7-*O*-glucuronide (OAG, purity over 95%) were supplied by Shanghai u-sea Biotech co., Ltd. (Shanghai China). 3,7-Dihydroxyflavone with purity of 97% was purchased from Indofine Chemical Company (Hillsborough, NJ, USA). Reference herb of *Radix Scutellariae* was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). HQ-Tab, C-HQ-Tab, YH-Cap, YH-DP, SHL-Cap, SHL-S-Cap were purchased from pharmacy stores in Mainland, China. Acetonitrile (Labscan Asia, Thailand) and methanol (TEDIA company, Inc., USA) were HPLC grade and used without further purification. All other reagents were of at least analytical grade. Distilled and deionized water was used throughout the experiment.

2.2. Instrumentations

The HPLC system consists of Waters 600 controller (pump), Waters 717 auto sampler and Waters 996 Photodiode Array UV detector. The separation of all the analytes was performed by using a Thermo BDS Hypersil column (250 mm × 4.6 mm; 5 μm particle size) connected to a guard column (Delta-Pak C18 Guard-Pak,

Waters). Data were collected by Waters Millennium software (version 3).

2.3. Chromatographic conditions

The mobile phase consisting of eluent A (20 mM sodium dihydrogen phosphate buffer, pH 4.6) and B (acetonitrile) was run at 1 ml/min. The linear gradient elution program was set as follows: eluent A decrease from 90 to 70% in the first 10 min, decrease from 70 to 40% in the next 2 min, maintained at 40% for 4 min, then eluent A increased from 40 to 90% from 16 to 20 min and equilibrated for 5 min before the next injection. The injection volume was 100 μl and the detective wavelength was set at 270 nm.

2.4. Preparation of standard solutions and calibration curves

The stock solution of B, BG, W, WG, OA and OAG (1 mg/ml) as well as the internal standard (2 mg/ml) were prepared by dissolving appropriate amount of each authentic compound in DMSO separately. The working solutions were prepared by mixing and diluting the stock solutions of each compound with methanol and phosphate buffer (20 mM, pH 2.5) comprising 1% ascorbic acid (50:50, v/v) to yield concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 μg/ml, respectively and the internal standard was diluted to the final concentration of 100 μg/ml.

Working solutions for calibration curves were prepared by mixing 0.7 ml of various working standard solutions with 20 μl of internal standard solution. The calibration curves were plotted by the peak-area ratio of each analyte/internal standard versus the concentrations of each analyte. To avoid the bias to low concentrations caused by the high concentrations of the standard curve, the calibration curves of each analyte was separated into two ranges: 0.02–0.5 and 0.5–10.0 μg/ml.

2.5. Sample preparation

To prepare samples from solid dosage forms, tablet, dripping pills, content from hard gelatin capsules were grinded to powder. To prepare samples from soft capsule, its content was obtained followed by mixing well into uniform semisolid. About 10 mg of the prepared powder or semisolid samples was accurately weighed and then extracted by 10 ml methanol:water mixture (80:20, v/v, containing 1 mM HCl). After sonication in water bath for 30 min, the mixture was centrifuged at 10,000 × *g* for 5 min. The supernatant was obtained followed by filtration with 0.45 μm syringe filter and dilution prior to injection into HPLC system for analysis.

2.6. Method validation

The linearity, intra-day and inter-day precision and accuracy were employed for method validation. The intra-day precision was determined within one day by analyzing five replicate control samples at concentrations of 0.05, 0.2, 1.0, 5.0 μg/ml. The inter-day precision was determined on five separate days for the control samples. The intra-day and inter-day precision was defined as the relative standard deviation (R.S.D.) and the accuracy was determined by calculating the relative error (R.E.). The limit of detection (LOD) was defined as the lowest concentration of the drug resulting in a signal-to-noise ratio of 3:1. The limit of quantification (LOQ) was defined as the lowest concentration of the drug resulting in a signal-to-noise ratio of 10:1 with precision below 20% and accuracy below ±20%.

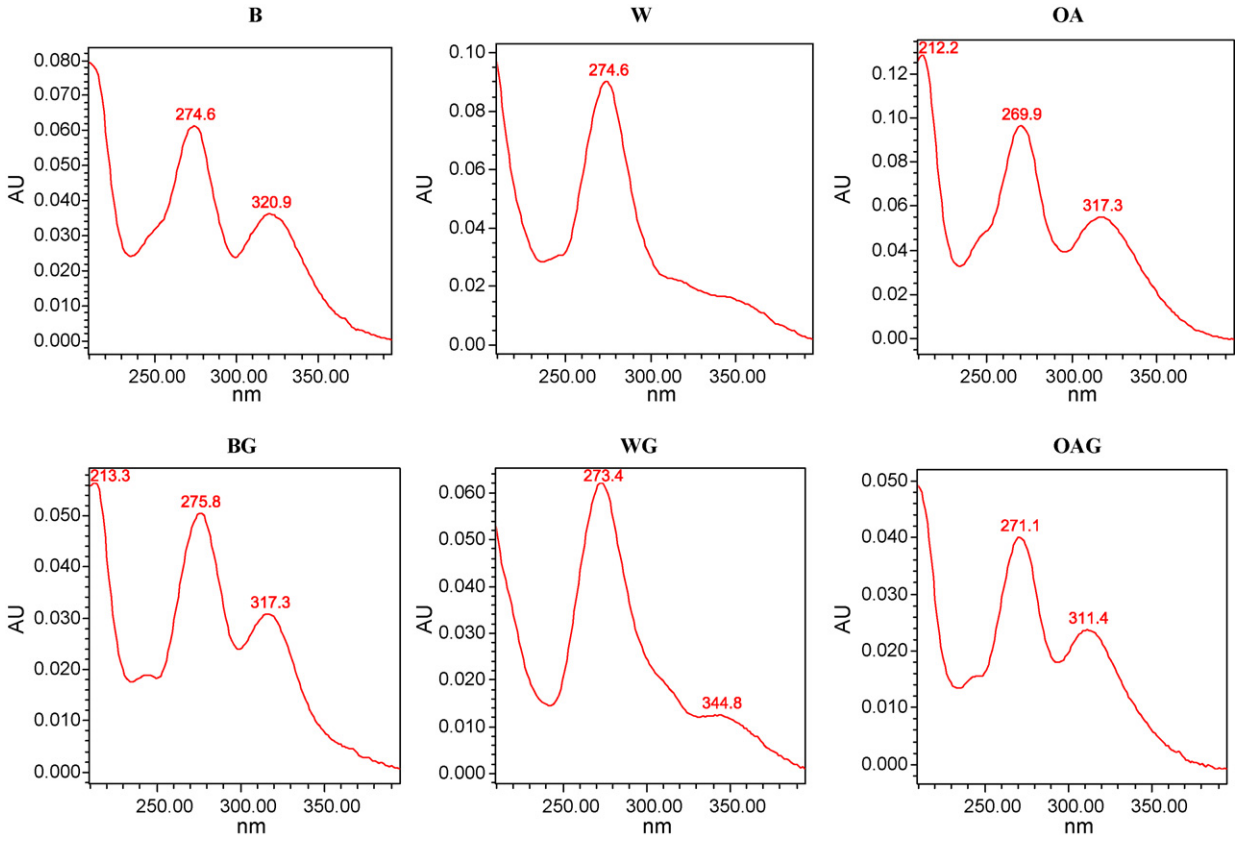

Fig. 2. UV spectra of major bioactive flavones in *Radix Scutellariae*.

Table 1
Regression equation, intra-day and inter-day precision and accuracy of six bioactive flavones.

Flavones	Linear range (μg/ml)	Regression equation	R^2	Nominal conc. (μg/ml)	Intra-day ($n = 5$)			Inter-day ($n = 5$)		
					Determined conc. (μg/ml)	Precision (%R.S.D.)	Accuracy (%R.E.)	Determined conc. (μg/ml)	Precision (%R.S.D.)	Accuracy (%R.E.)
BG	0.02	$y = 0.8354X - 0.0019$	0.9998	0.05	0.049 ± 0.001	2.619	−1.843	0.049 ± 0.001	1.28	−2.506
	–0.50			0.2	0.195 ± 0.003	1.327	−2.322	0.200 ± 0.004	2.008	−0.174
	0.50	$y = 0.7982X - 0.0025$	1	1.0	1.001 ± 0.006	0.594	0.113	0.990 ± 0.036	3.625	−0.981
	–10.0			5.0	5.105 ± 0.073	1.432	2.110	5.106 ± 0.171	3.357	2.121
WG	0.02	$y = 1.0562X - 0.0036$	0.9998	0.05	0.049 ± 0.002	3.731	−2.119	0.049 ± 0.001	1.412	−1.397
	–0.50			0.2	0.197 ± 0.001	0.439	−1.621	0.201 ± 0.004	1.930	0.526
	0.50	$y = 1.0290X - 0.0180$	0.9999	1.0	0.976 ± 0.006	0.620	−2.355	0.965 ± 0.037	3.798	−3.518
	–10.0			5.0	5.107 ± 0.078	1.532	2.132	5.106 ± 0.169	3.307	4.057
OAG	0.02	$y = 0.7811X - 0.0009$	0.9998	0.05	0.050 ± 0.002	3.856	−0.643	0.050 ± 0.000	0.466	−0.419
	–0.50			0.2	0.195 ± 0.002	0.786	−2.299	0.202 ± 0.007	3.600	1.085
	0.50	$y = 0.7579X - 0.0059$	0.9999	1.0	0.982 ± 0.014	1.414	−1.794	0.983 ± 0.042	4.271	−1.656
	–10.0			5.0	5.193 ± 0.130	2.510	3.850	5.203 ± 0.180	3.451	4.057
B	0.02	$y = 1.1092X + 0.002$	0.9975	0.05	0.047 ± 0.002	4.170	−6.142	0.049 ± 0.002	4.712	−2.735
	–0.50			0.2	0.193 ± 0.001	0.489	−3.296	0.199 ± 0.004	2.085	−0.579
	0.50	$y = 1.1866X - 0.0308$	0.9999	1.0	0.966 ± 0.008	0.848	−3.414	0.945 ± 0.042	4.424	−5.522
	–10.0			5.0	5.170 ± 0.058	1.127	3.408	5.198 ± 0.168	3.238	3.965
W	0.02	$y = 1.3771X - 0.002$	0.9994	0.05	0.049 ± 0.001	2.706	−1.726	0.049 ± 0.003	5.894	−2.493
	–0.50			0.2	0.193 ± 0.001	0.740	−3.261	0.201 ± 0.010	4.758	0.538
	0.50	$y = 1.3615X - 0.0895$	0.9982	1.0	1.033 ± 0.011	1.104	3.251	0.922 ± 0.090	9.793	−7.834
	–10.0			5.0	5.172 ± 0.059	1.132	3.433	5.191 ± 0.200	3.859	3.814
OA	0.02	$y = 1.7977X + 0.0125$	0.9998	0.05	0.048 ± 0.002	4.976	−3.039	0.052 ± 0.005	10.139	3.236
	–0.50			0.2	0.197 ± 0.004	1.781	−1.350	0.202 ± 0.004	1.861	1.192
	0.50	$y = 1.7787X + 0.0256$	0.9999	1.0	0.968 ± 0.010	1.061	−3.191	0.945 ± 0.064	3.739	−5.536
	–10.0			5.0	5.075 ± 0.058	1.143	1.501	5.105 ± 0.163	3.198	2.105

2.7. Extraction recovery

Samples of the *Radix Scutellariae* reference herb and PTCM products (HQ-Tab, C-HQ-Tab, YH-Cap, YH-DP, SHL-Cap, SHL-S-Cap) were obtained and divided into two equal parts. One part was spiked with three different concentrations of standard solutions (low, medium and high) followed by thorough mixing and extraction as described in Section 2.5, whereas the other part of the sample was directly

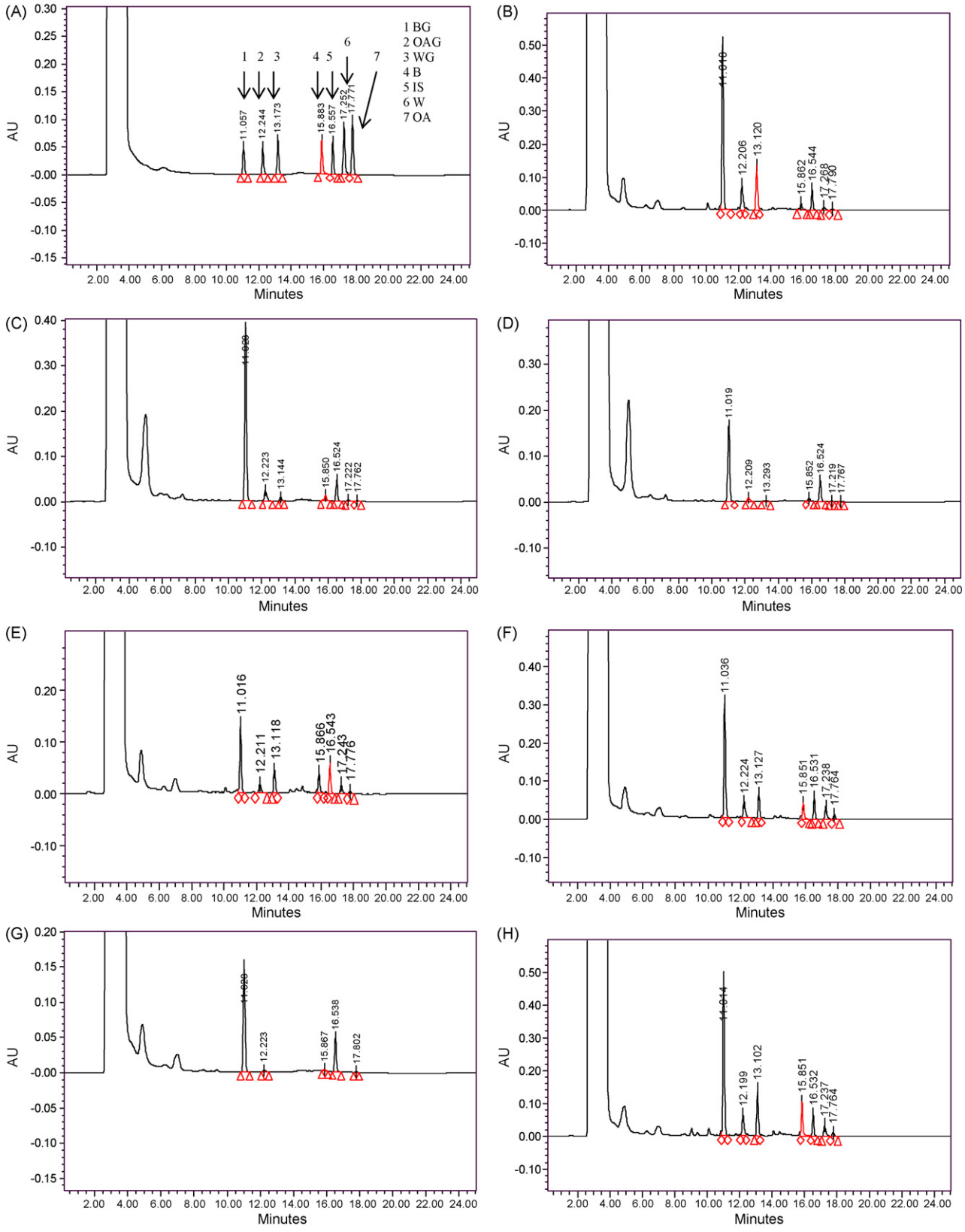

Fig. 3. Representative HPLC chromatograms of standard solution and extract of *Radix Scutellariae* (A) standard solution at 1 μg/ml; (B) reference herb; (C) SHL-Cap; (D) SHL-S-Cap; (E) C-HQ-Tab; (F) HQ-Tab; (G) YH-DP; (H) YH-Cap.

extracted as described in Section 2.5. The extraction recoveries of each compound were calculated as the percentage of the net amount of each compound obtained (determined amount in spiked samples – determined amount in un-spiked sample) after extraction versus actual amount added.

2.8. Application

The developed assay was applied to simultaneously determine the contents of baicalein, baicalin, wogonin, wogonoside, oroxylin A and oroxylin A-7-*O*-glucuronide in the reference herb and the commercial PTCM of *Radix Scutellariae* in forms of tablet, capsule, dripping pill and soft capsule. All the samples were treated as described in Section 2.5 followed by HPLC analysis. Since the contents of each analyte may vary in different products, the extracted solutions were appropriately diluted in order to fit the concentration range of the calibration curve.

3. Results and discussions

3.1. Chromatography

A simple HPLC/UV method has been established and validated for simultaneous determination of the content of six representative flavones in the *Radix Scutellariae*. Due to the difference in the polarity between the glycosides and aglycones, it is difficult to separate them in an isocratic elution. Thus, a linear gradient elution program has been employed.

Fig. 2 exhibits the UV spectra of the six bioactive flavones. Fig. 3 shows the representative HPLC chromatograms of the standard solution of six analytes at 1 μg/ml, reference herb of *Radix Scutellariae* and the six PTCM products containing *Radix Scutellariae*. Under the current chromatographic condition, the six components were satisfactorily separated. It was demonstrated that the co-existing ingredients in *Radix Scutellariae* did not interfere in the determination of the six analytes and the internal standard. The peak of each analyte was identified by the comparison of the retention time and UV spectra with the authentic standards and confirmed by LC/MS/MS.

3.2. Method validation

The results of linear range and regression equations for the calibration curves are shown in Table 1. The calibration curves were plotted by the peak-area ratio of each analyte to the internal standard versus the concentration of each analyte. To avoid the bias to the low concentrations of the standard curve by the high concentrations, the calibration curves were separated into two ranges, 0.02–0.5 and 0.5–10.0 μg/ml. Within the linear range, the calibration curve had good linearity ($R^2 > 0.99$) for each analyte. For all the six bioactive flavones, LOD was 0.01 μg/ml and the LOQ was 0.02 μg/ml. The intra-day and inter-day precision and accuracy of the assay method are also shown in Table 1. The R.S.D. of both the intra-day and inter-day precision for all the six compounds was below 10.139%. The accuracy at four different concentrations was within the range of −7.834 to 4.057%.

3.3. Extraction efficiency

The 2005 edition of Chinese pharmacopeia provides a method for the quality control of *Radix Scutellariae* by determining the content of baicalin and 9% is the lowest content requirement in which 70% ethanol was employed as extraction solvent [34]. For the current study, considering the higher solubility of the flavones in methanol and the convenience of operation, methanol was used as extraction solvent and sonication was employed to do the extraction.

Four different extraction solvents including methanol, methanol:water (80:20), methanol:water (50:50), methanol:water (20:80) have been tried to extract the bioactive flavones from the reference herb and C-HQ-Tab, the representative PTCM product of *Radix Scutellariae*. The extraction solvent of methanol:water (80:20) produced the highest extraction efficiency for the reference herb of *Radix Scutellariae*. It was found that the extraction solvent system have less impact on the extraction efficiency of the PTCM product than that in the reference herb, which may be due to that the commercial products are produced by diluting the prepared *Radix Scutellariae* extract with other excepient and are generally in low content of each component. Subsequently, the solvent of methanol:water (80:20) containing 1 mM HCl was employed throughout the experiment in order to optimize the unionized portion of the studied weak acidic components for their extraction by methanol. Then the efficiency of extraction method was tested in different dosage forms of the commercial products, i.e. HQ-Tab, C-HQ-Tab, YH-Cap, YH-DP, SHL-Cap, SHL-S-Cap and the reference herb of *Radix Scutellariae*. The result of extraction recovery is listed in Table 2. The mean extraction recovery for the six bioactive

Table 2a
Extraction recoveries of BG, WG, OAG in various products of *Radix Scutellariae* ($n = 3$).

Product	BG			WG			OAG		
	Conc. (μg/ml)			Conc. (μg/ml)			Conc. (μg/ml)		
	Spiked	Detected	Recovery (%)	Spiked	Detected	Recovery (%)	Spiked	Detected	Recovery (%)
RH	2.0	1.79 ± 0.03	89.43 ± 1.72	2.0	1.88 ± 0.03	94.07 ± 1.56	2.0	1.87 ± 0.06	93.28 ± 2.78
	5.0	4.36 ± 0.07	102.25 ± 1.49	5.0	4.88 ± 0.07	97.61 ± 0.36	5.0	4.75 ± 0.15	94.98 ± 2.96
	10.0	9.54 ± 0.20	95.42 ± 1.95	10.0	9.51 ± 0.10	95.12 ± 0.98	10.0	9.42 ± 0.13	94.16 ± 1.33
HQ-Tab	2.0	1.94 ± 0.06	96.78 ± 2.83	1.0	1.00 ± 0.01	100.41 ± 0.71	1.0	1.01 ± 0.01	101.15 ± 1.15
	5.0	4.96 ± 0.16	99.17 ± 3.21	2.0	2.06 ± 0.03	102.87 ± 1.46	2.0	2.06 ± 0.02	103.07 ± 1.22
	10.0	9.54 ± 0.01	95.42 ± 0.11	5.0	5.33 ± 0.01	106.57 ± 0.13	5.0	5.26 ± 0.02	105.26 ± 0.34
YH-DP	2.0	1.97 ± 0.07	98.60 ± 3.61	0.20	0.21 ± 0.00	104.42 ± 0.30	0.20	0.21 ± 0.00	107.33 ± 1.67
	5.0	4.66 ± 0.02	93.19 ± 0.33	0.50	0.52 ± 0.01	104.23 ± 2.75	0.50	0.49 ± 0.03	97.26 ± 5.55
	10.0	9.80 ± 0.07	97.99 ± 0.69	1.00	1.04 ± 0.00	103.90 ± 0.09	1.00	1.02 ± 0.02	102.30 ± 1.68
YH-Cap	2.0	2.05 ± 0.02	102.68 ± 1.03	1.0	0.99 ± 0.01	98.73 ± 0.61	1.0	1.03 ± 0.04	103.04 ± 4.07
	5.0	4.87 ± 0.02	97.39 ± 0.44	2.0	1.96 ± 0.08	98.12 ± 4.14	2.0	2.11 ± 0.05	105.36 ± 2.34
	10.0	9.94 ± 0.02	99.39 ± 0.23	5.0	4.59 ± 0.04	91.80 ± 0.75	5.0	5.03 ± 0.01	100.57 ± 0.15
SHL-S-Cap	2.0	1.92 ± 0.07	95.80 ± 3.58	0.1	0.09 ± 0.00	90.15 ± 3.35	0.2	0.20 ± 0.00	101.12 ± 2.19
	5.0	4.60 ± 0.04	92.07 ± 0.79	0.2	0.19 ± 0.00	96.24 ± 1.04	0.5	0.50 ± 0.01	100.75 ± 1.16
	10.0	9.41 ± 0.05	94.09 ± 0.55	1.0	0.92 ± 0.00	91.83 ± 0.33	1.0	0.94 ± 0.01	94.46 ± 1.20

Table 2b
Extraction recoveries of B, W, OA in various products of *Radix Scutellariae* ($n = 3$).

Products	B			W			OA		
	Conc. (μg/ml)			Conc. (μg/ml)			Conc. (μg/ml)		
	Spiked	Detected	Recovery (%)	Spiked	Detected	Recovery (%)	Spiked	Detected	Recovery (%)
RH	0.5	0.53 ± 0.01	105.69 ± 1.11	0.5	0.48 ± 0.02	95.94 ± 4.00	0.5	0.47 ± 0.02	93.59 ± 3.09
	1.0	1.01 ± 0.02	101.18 ± 2.49	1.0	0.93 ± 0.04	93.05 ± 4.18	1.0	0.97 ± 0.04	97.26 ± 3.59
	2.0	2.01 ± 0.08	100.32 ± 4.22	2.0	2.15 ± 0.01	107.30 ± 0.56	2.0	2.02 ± 0.02	101.24 ± 1.23
HQ-Tab	1.0	0.97 ± 0.01	97.40 ± 0.68	1.0	0.97 ± 0.03	97.10 ± 2.89	0.5	0.52 ± 0.01	104.15 ± 2.82
	2.0	2.06 ± 0.03	102.96 ± 1.68	2.0	1.99 ± 0.03	99.27 ± 1.45	1.0	0.97 ± 0.01	97.09 ± 0.90
	5.0	4.71 ± 0.20	94.14 ± 4.07	5.0	5.00 ± 0.17	100.08 ± 3.44	2.0	2.06 ± 0.06	102.99 ± 3.22
YH-DP	0.2	0.20 ± 0.00	102.22 ± 1.33	0.2	0.18 ± 0.01	91.79 ± 3.29	0.1	0.10 ± 0.00	104.77 ± 1.03
	0.5	0.50 ± 0.02	101.00 ± 3.89	0.5	0.49 ± 0.02	97.12 ± 3.04	0.2	0.20 ± 0.01	101.60 ± 5.12
	1.0	0.96 ± 0.02	96.26 ± 1.71	1.0	1.03 ± 0.02	102.75 ± 1.53	0.5	0.49 ± 0.03	98.46 ± 5.15
YH-Cap	1.0	0.98 ± 0.01	97.89 ± 0.82	0.2	0.19 ± 0.01	95.96 ± 4.89	0.2	0.21 ± 0.01	105.09 ± 4.79
	2.0	1.88 ± 0.08	93.87 ± 4.19	0.5	0.46 ± 0.01	92.88 ± 0.93	0.5	0.52 ± 0.02	103.34 ± 4.24
	5.0	4.70 ± 0.13	93.97 ± 2.64	1.0	1.04 ± 0.04	103.69 ± 3.51	1.0	1.02 ± 0.04	102.16 ± 3.71
SHL-S-Cap	0.2	0.19 ± 0.01	96.69 ± 5.10	0.1	0.09 ± 0.00	89.22 ± 2.07	0.1	0.09 ± 0.01	93.09 ± 4.80
	0.5	0.48 ± 0.01	95.11 ± 2.07	0.2	0.19 ± 0.01	93.39 ± 2.45	0.2	0.18 ± 0.00	90.84 ± 0.64
	1.0	1.04 ± 0.01	104.35 ± 1.37	1.0	0.97 ± 0.01	97.00 ± 0.70	1.0	0.89 ± 0.01	89.89 ± 0.80

Table 3
Contents of six bioactive flavones in reference herb and commercial PTCM products of *Radix Scutellariae*.

Products	Content (n = 3, mean ± S.D., mg/100 mg)					
	BG	WG	OAG	B	W	OA
SHL-Cap	16.32 ± 0.07	0.23 ± 0.01	1.19 ± 0.04	0.39 ± 0.01	0.10 ± 0.01	0.03 ± 0.00
SHL-S-Cap	6.19 ± 0.04	0.03 ± 0.00	0.35 ± 0.01	0.22 ± 0.00	0.04 ± 0.00	0.02 ± 0.00
YH-Cap	7.72 ± 0.01	1.78 ± 0.01	1.27 ± 0.01	1.22 ± 0.02	0.39 ± 0.00	0.09 ± 0.00
YH-DP	10.52 ± 0.12	N.D.	0.22 ± 0.01	0.15 ± 0.00	N.D.	0.01 ± 0.00
HQ-Tab	4.59 ± 0.08	0.81 ± 0.01	0.84 ± 0.01	0.64 ± 0.01	0.39 ± 0.01	0.10 ± 0.01
C-HQ-tab	2.25 ± 0.01	0.67 ± 0.02	0.38 ± 0.01	0.53 ± 0.02	0.19 ± 0.02	0.04 ± 0.00
RH	18.54 ± 0.71	3.54 ± 0.18	2.84 ± 0.14	0.53 ± 0.01	0.21 ± 0.01	0.06 ± 0.00

N.D.: not detectable.

flavones in five commercial products was within the range of 89.22–107.33%.

3.4. Application

Due to the co-existing multiple bioactive components in the traditional Chinese medicinal products, it is far from enough to just monitor one component for the quality control of its raw material and proprietary traditional Chinese medicine products. Therefore, current study developed a simple and accurate assay method for simultaneous determination of six major bioactive flavones in *Radix Scutellariae*.

The validated HPLC/UV method was applied to the simultaneous determination of baicalein, baicalin, wogonin, wogonoside, oroxylin A and oroxylin A-7-*O*-glucuronide in the reference herb of *Radix Scutellariae* as well as six commercial products containing *Radix Scutellariae*, i.e. HQ-Tab, C-HQ-Tab, YH-Cap, YH-DP, SHL-Cap, SHL-S-Cap. The results are shown in Table 3.

The content of BG under our extraction and determination method existed in the most abundant amount comparing with the other five bioactive flavones and complied with the requirement of the pharmacopeia and the literature report [34,35,37,39]. Furthermore, the content of each studied flavones showed marked variations among different dosage forms. The amount of glycosides (BG, WG, OAG) ranked the highest in the reference herb, and the YH-Cap contained the highest amount of aglycones (B, W, OA). W and WG could not be detected in the dripping pill. In addition, the contents of the aglycones are the least among in all the products tested. This might result from the raw material that has been used or the procedure of extraction and pharmaceutical preparation of different dosage forms. It has been observed that the content of each flavone in the raw material of *Radix Scutellariae* from different areas showed a great extent of variation [43–45] and the content of BG in plants from some areas was even below 9%. In the cases of YH-DP and SHL-S-Cap, the variances in solubility of different flavones in the formulation bases might cause the content variances in the final products. Particularly, HQ-Tab and C-HQ-Tab were extracted without removing the sugar coating and the content of the six flavones were calculated with the weight of the sugar coating, which might contribute to the relatively low amount of the flavones detected.

4. Conclusion

In the present study, a simple and accurate HPLC/UV method has been established and validated to simultaneously determine six main bioactive flavones (baicalein, baicalin, wogonin, wogonoside, oroxylin A and oroxylin A-7-*O*-glucuronide) in the reference herb and commercial PTCM of *Radix Scutellariae*. It produced good linearity, intra-day and inter-day precision and accuracy as well as high extraction efficiency. The assay method is helpful to improve the quality control of authentic material of *Radix Scutellariae* and its proprietary traditional Chinese medicines.

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