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Short communication

Simultaneous determination of eight active components in Chinese medicine 'JiangYaBiFeng' tablet by HPLC coupled with diode array detection

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ABSTRACT

An effective, accurate and reliable method was developed for the simultaneous separation and determination of eight active components (baicalin, baicalein, sophoricoside, rutin, quercetin, genistein, pargyline and hydrochlorothiazide) in Chinese medicine 'JiangYaBiFeng' tablet (JYBF tablet) by high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD). Due to the different UV characteristic of these components, different wavelengths were selected for analysis of different analytes, such as 210 nm for pargyline, 256 nm for sophoricoside, rutin, quercetin and genistein, and 280 nm for baicalin, baicalein and hydrochlorothiazide. Excellent linear behaviors over the investigated concentration ranges were observed with the values of R^2 higher than 0.9990 for all analytes. The recovery rates and relative standard deviation (RSD) for all analytes at three different concentrations were 94.9–104.7% and 1.23–3.00%, respectively. The validated method was successfully applied to the simultaneously determination of these active components in 'JiangYaBiFeng' tablet from different production batches, indicating that the proposed method in this paper was particularly suitable for the routine analysis of JYBF tablet and its quality control.

1. Introduction

'JiangYaBiFeng' tablet (JYBF tablet), composed of two western medicines (pargyline and hydrochlorothiazide) and three traditional Chinese medicines (TCMs) (*Scutellaria baicalensis*, *Sophora japonica*, and *Arachis hypogaea*), is a new drug for the treatment of hypertension. The pharmacological studies have revealed that flavones were the major active components of these above TCMs and played important roles in the treatment of hypertension [1–6]. Therefore, it could be expected that flavones and the two western medicines were the active components and responsible for the therapeutic effects of JYBF tablet [7–10].

In Chinese medicines, some active components frequently were usually regarded as index component for quality control in their Chinese prescriptions. However, up to now, only hydrochlorothiazide was chosen as index component for quality control of JYBF tablet [11]. Therefore, in order to further effectively utilize it and enhance the clinical safety, an accurate and reliable method based on the multiple constituents is urgently needed to develop for quality control of JYBF tablet. However, to our knowledge, the method for the simultaneous separation and determination of multiple active components in JYBF tablet by HPLC has not been found.

In this study, a simple, accurate and reliable analytical method for the simultaneous determination of eight active components (including baicalin, baicalein, sophoricoside, rutin, quercetin, genistein, pargyline and hydrochlorothiazide) in JYBF tablet was developed by HPLC method coupled with diode array detection (DAD). From these results, the proposed method in this paper is particularly suitable for the routine analysis of JYBF tablet and its quality control.

2. Experimental

2.1. Chemicals and reagents

JYBF tablet was supplied by Chinese pharmaceutical manufacturer (Zhongxin, Tianjin, China). Baicalin, baicalein, sophoricoside, rutin, quercetin and genistein were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Pargyline and hydrochlorothiazide were purchased from Guangdong Guanghua Chemical Factory (Guangzhou, China). Acetonitrile and methanol were of HPLC grade, and purchased from Shield Fine Chemicals Company (Tianjin, China). Ammonium dihydrogen phosphate and phosphoric acid were ana-

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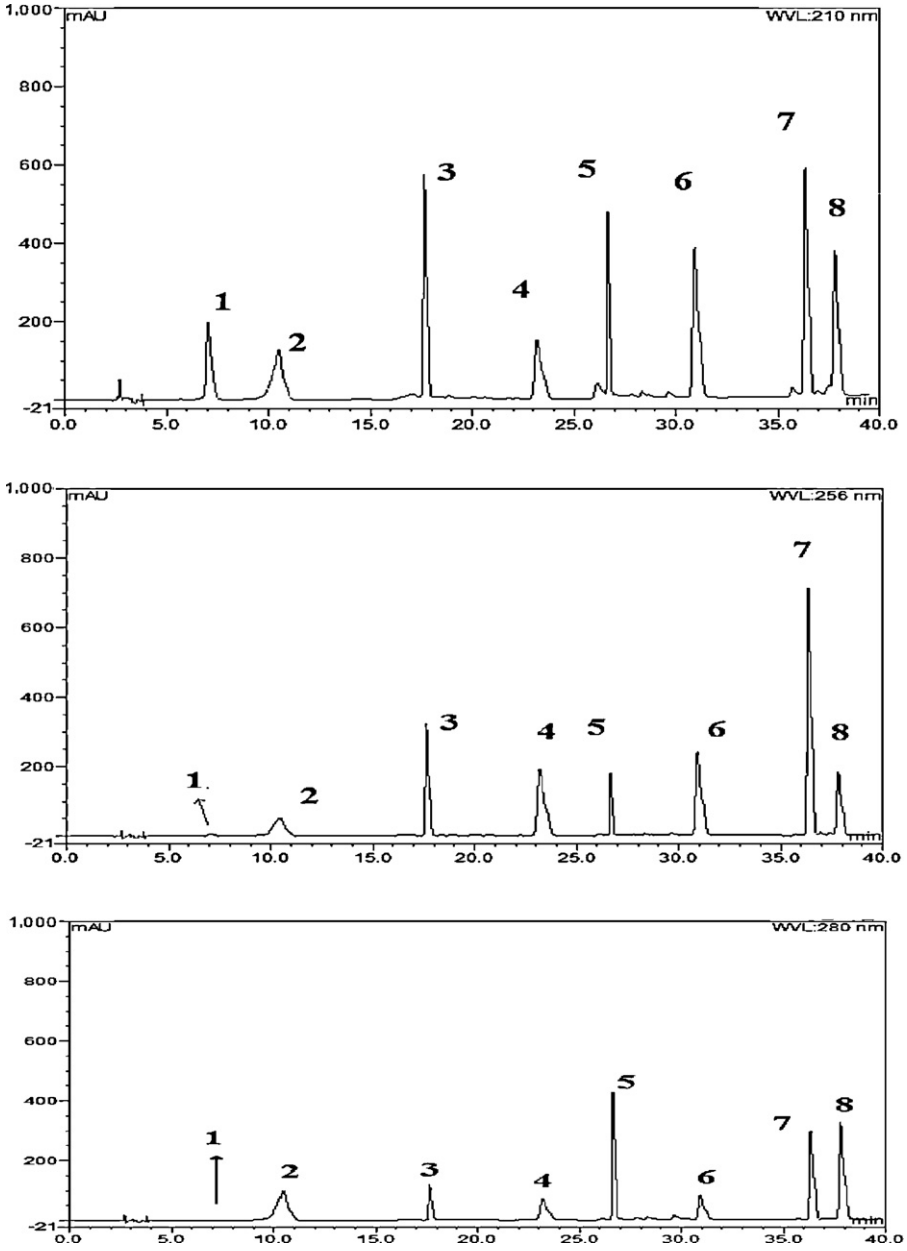

Fig. 1. Representative chromatograms of standard solutions (a, 210 nm; b, 256 nm; c, 280 nm). 1 – Pargyline, 2 – hydrochlorothiazide, 3 – sophoricoside, 4 – rutin, 5 – baicalin, 6 – quercetin, 7 – genistein, 8 – baicalein.

lytical reagents and both were purchased from Damao Chemical reagent Factory (Tianjin, China). Other reagents were all of analytical grade. Deionized water used throughout the experiments was generated by a Milli-Q academic water purification system (Shanghai, China).

2.2. Instrumentation and analytical conditions

The HPLC system Dionex P680 series (Dionex, USA), equipped with the Chromeleon software (Dionex) and comprised a binary pump, an online vacuum degasser, a manual sampler, a thermostated column compartment and a diode array detection (DAD), was used for the chromatographic analysis. All separations were carried out on a DIONEX Acclaim C_{18} column (250 mm × 4.6 mm, 5.0 μm). A linear gradient elution of eluents A (50 mM ammonium phosphate buffer, pH 3.0) and B (acetonitrile) was used to run the separation. The elution programme was well optimized and conducted as follows: an isocratic elution of 15–22% B with the first 12 min, a linear gradient of 22–33% B with the range of 12–22 min, 33–43% B with the range of 22–32 min and then an isocratic elution of 43% B for the next 10 min. After holding the solvent composition of 43% B for a further minute the column was returned to its starting condition. The solvent flow rate was 1.0 mL min^{-1}, the injection volume was 20 μL, and the column temperature was maintained at 25 °C.

2.3. Standard preparation

The standard stock solutions of baicalin (0.50 mg mL^{-1}), baicalein (0.45 mg mL^{-1}), sophoricoside (0.44 mg mL^{-1}), rutin (0.50 mg mL^{-1}), quercetin (0.50 mg mL^{-1}), genistein (0.50 mg mL^{-1}), pargyline (0.50 mg mL^{-1}) and hydrochloroth-

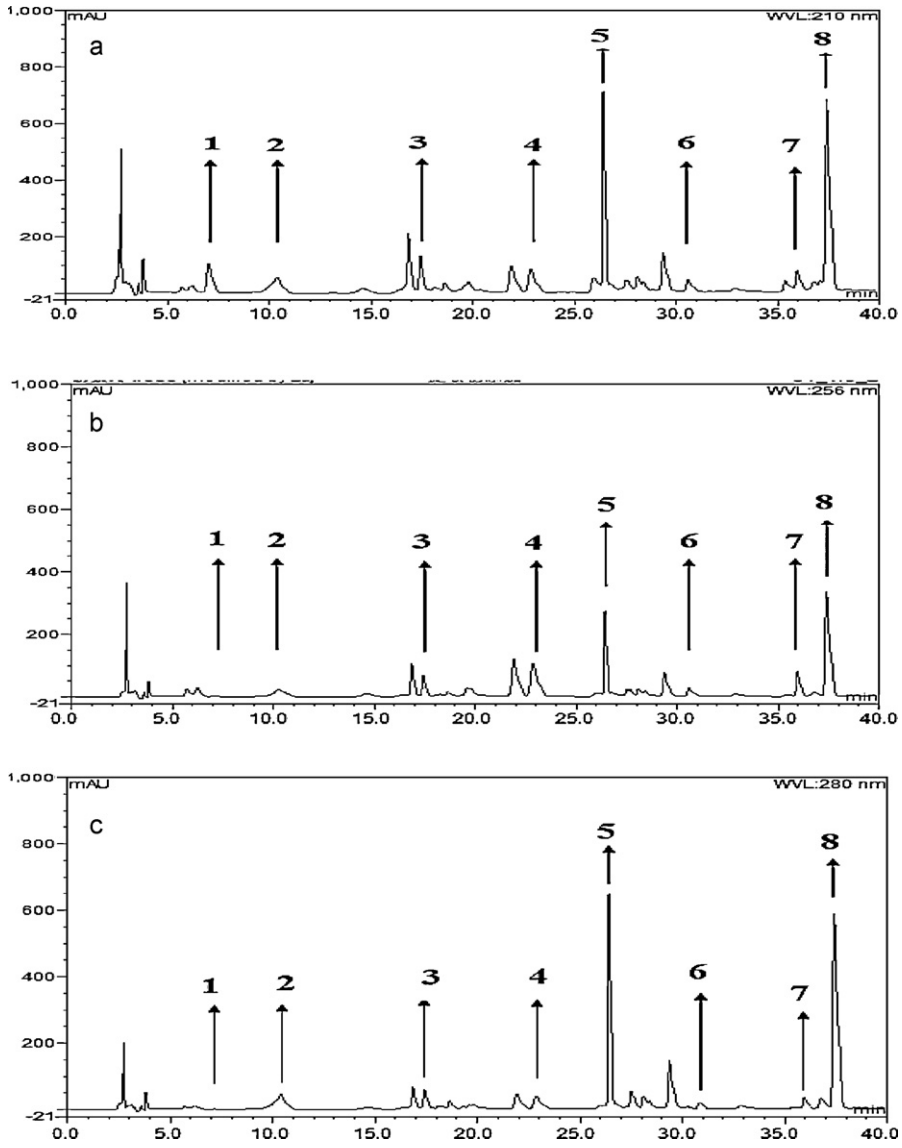

Fig. 2. Representative chromatograms of sample solution (a, 210 nm; b, 256 nm; c, 280 nm). 1 – Pargyline, 2 – hydrochlorothiazide, 3 – sophoricoside, 4 – rutin, 5 – baicalin, 6 – quercetin, 7 – genistein, 8 – baicalein.

iazide (0.50 mg mL^{-1}) were prepared in methanol and stored away from light at 4 °C. Working solutions were prepared by appropriate dilution of the stock solution.

2.4. Sample preparation

Grind 10 pills of JYBF tablet into powder by use of a pestle and mortar and 0.15 g of powder was weighed accurately and added into a calibrated flask with 100 mL methanol. The mixture then was suspended in an ultrasonic bath for 30 min and filtered through a 0.22 μm membrane and analyzed by HPLC.

3. Results and discussion

3.1. Chromatographic separation

Considering the total resolution of the chromatographic separation, the running time and solvent/reagent consumption, the

Table 1
Calibration curves, LOD and LLOQ of eight active components.

Analytes	Calibration curves	R^2	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LLOQ (μg mL^{-1})
Sophoricoside	$y = 0.8767x - 0.2308$	0.9992	0.20–62.50	0.04	0.13
Rutin	$y = 1.1669x - 0.1947$	0.9995	0.10–62.50	0.01	0.04
Baicalin	$y = 0.7945x + 0.2019$	0.9992	0.20–100.00	0.05	0.15
Quercetin	$y = 1.1528x - 0.0404$	0.9994	0.13–62.50	0.03	0.10
Genistein	$y = 2.4040x - 0.3048$	0.9991	0.20–50.00	0.05	0.13
Baicalein	$y = 1.5459x - 0.2138$	0.9990	0.20–100.00	0.02	0.07
Pargyline	$y = 1.0197x + 5.4056$	0.9995	0.20–50.00	0.05	0.16
Hydrochlorothiazide	$y = 0.5114x - 0.1227$	0.9996	0.20–62.50	0.04	0.14

Table 2
Precision, repeatability and stability of the method ($n = 6$).

Analytes	Concentration ($\mu g\,mL^{-1}$)	Precision		Repeatability RSD (%)	Stability RSD (%)
		Intra-day RSD (%)	Inter-day RSD (%)		
Sophoricoside	22.00	0.46	1.08	1.23	2.56
Rutin	25.00	1.05	2.25	2.47	2.77
Baicalin	25.00	0.17	1.21	0.98	2.08
Quercetin	25.00	0.24	1.88	1.82	1.83
Genistein	25.00	0.22	1.16	1.18	2.01
Baicalein	28.00	0.58	2.17	2.29	2.52
Pargyline	25.00	0.81	2.09	2.38	2.41
Hydrochlorothiazide	25.00	0.49	1.74	2.06	1.96

mobile phase composed of acetonitrile-ammonium phosphate buffer (pH 3.0) was chosen for the separation. Because of the different UV characteristic of analytes investigated, different wavelengths were selected for analysis of different analytes, such as 210 nm for pargyline, 256 nm for sophoricoside, rutin, quercetin, genistein, and 280 nm for baicalin, baicalein, hydrochlorothiazide. The typical chromatographic profiles of the standard solution and the real sample solution were shown in Figs. 1 and 2, respectively. It was indicated that a good separation was obtained under the described condition and no interfering peaks were found at the retention time of analytes.

3.2. Calibration curve and limit of detection

Calibration curves were constructed by plotting the peak area against the corresponding concentration of the standard solutions. The injection concentration, which could be detected at the signal-to-noise ratio of 3 (S/N = 3), was considered to be the limit of detection (LOD). The lower limit of quantification (LLOQ) was the injection concentration corresponding to the peak heights with signal-to-noise ratio of 10 (S/N = 10). The detailed descriptions of the regression curves were presented in Table 1.

3.3. Precision, repeatability, stability and accuracy

The intra- and inter-day assay precisions were repetitively carried out on the samples (baicalin, 25.00 $\mu g\,mL^{-1}$; baicalein, 28.00 $\mu g\,mL^{-1}$; sophoricoside, 22.00 $\mu g\,mL^{-1}$; rutin, 25.00 $\mu g\,mL^{-1}$; quercetin, 25.00 $\mu g\,mL^{-1}$; genistein, 25.00 $\mu g\,mL^{-1}$; pargyline, 25.00 $\mu g\,mL^{-1}$; hydrochlorothiazide, 25.00 $\mu g\,mL^{-1}$) six times a day and once a day for six sequential days, respectively. The injection repeatability was determined by the injection of continuous six times using the same sample, while the analysis repeatability was examined by the injection of six different samples, which were prepared with the same sample preparation procedure. The precision and repeatability of the solution at medium concentration were shown in Table 2. For the stability testing, the same real sample was analyzed within 24 h at the room temperature. The stabilities of the solution shown in RSD of retention time and peak area were all within ±3% and no significant difference was observed, indicating that the solution was stable (shown in Table 2).

Accuracy was defined as the rate of the calculated value by the standard curve to that of its true value, expressed as recovery rate (%). The recovery rate and RSD for analytes at different concentrations were shown in Table 3.

Table 3
Recovery of the method ($n = 3$).

Analytes	Added ($\mu g\,mL^{-1}$)	Found ($\mu g\,mL^{-1}$)	Recovery rate (%)	RSD (%)
Sophoricoside	4.54	4.64	102.3	2.96
	5.67	5.42	95.6	2.08
	6.80	6.73	98.9	1.34
Rutin	10.54	10.72	101.7	1.28
	13.17	13.72	104.2	2.14
	15.80	15.58	98.6	1.23
Baicalin	31.49	29.88	94.9	1.67
	39.36	39.01	99.1	2.96
	47.23	49.45	104.7	2.56
Quercetin	0.18	0.17	95.3	1.89
	0.23	0.23	100.3	2.04
	0.28	0.29	103.4	2.33
Genistein	0.39	0.39	99.8	1.28
	0.50	0.49	98.7	3.00
	0.60	0.63	104.2	1.45
Baicalein	39.62	37.64	95.0	2.78
	49.52	50.86	102.7	1.98
	59.42	57.34	96.5	1.39
Pargyline	7.61	7.40	97.3	1.02
	9.51	9.88	103.9	2.47
	11.41	11.20	98.2	2.38
Hydrochlorothiazide	11.50	11.59	100.8	2.19
	14.37	14.24	99.1	1.66
	17.25	16.70	96.8	2.33

Table 4
Determination of the eight active components in 'JiangYaBiFeng' tablets by the HPLC method.

Analytes	Content ($mg\,g^{-1}$) (Batch no. 0606916)			Content ($mg\,g^{-1}$) (Batch no. 0908560)		
	1[a]	2[a]	3[a]	1[b]	2[b]	3[b]
Pargyline	6.34 ± 0.08	6.31 ± 0.11	6.44 ± 0.12	6.37 ± 0.08	6.51 ± 0.13	6.45 ± 0.09
Hydrochlorothiazide	9.58 ± 0.07	9.67 ± 0.06	9.72 ± 0.11	9.55 ± 0.08	9.60 ± 0.06	9.46 ± 0.09
Sophoricoside	3.78 ± 0.05	3.56 ± 0.06	3.62 ± 0.04	3.90 ± 0.03	4.11 ± 0.05	4.03 ± 0.04
Rutin	8.78 ± 0.11	9.02 ± 0.12	8.55 ± 0.11	8.25 ± 0.10	8.33 ± 0.13	8.18 ± 0.11
Baicalin	26.24 ± 0.13	25.94 ± 0.11	25.66 ± 0.15	22.39 ± 0.12	22.58 ± 0.12	22.72 ± 0.12
Quercetin	0.15 ± 0.02	0.12 ± 0.03	0.11 ± 0.04	0.17 ± 0.04	0.16 ± 0.02	0.19 ± 0.05
Genistein	0.33 ± 0.03	0.37 ± 0.05	0.31 ± 0.04	0.42 ± 0.03	0.45 ± 0.07	0.39 ± 0.06
Baicalein	33.01 ± 0.17	34.26 ± 0.15	33.47 ± 0.14	36.39 ± 0.14	36.54 ± 0.15	36.78 ± 0.16

[a] Indicate the different samples from the Batch no. 0606916.
[b] Indicate the different samples from the Batch no. 0908560.

3.4. Application

In order to examine the application for practical analysis, the proposed method was applied to the simultaneous determination of eight active components in JYBF tablet with different production batches. The results were summarized in Table 4, indicating that the concentrations of analytes existed a little difference between different production batches. Therefore, it is very significant to develop an effective, accurate and reliable method for analysis of JYBF tablet. The proposed method in this paper is particularly suitable for the routine analysis of JYBF tablet and its quality control.

4. Conclusion

In this study, an accurate and reliable analytical method for the simultaneous determination of eight active components (baicalin, baicalein, sophoricoside, rutin, quercetin, genistein, pargyline and hydrochlorothiazide) in JYBF tablet was developed by RP-HPLC method coupled with UV detection. The proposed method is promising to be the routine analysis for JYBF tablet and its quality control with simplicity, accuracy and reliability.

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