Comparison of Five Prescriptions Composed by Coptidis Rhizoma and Evodiae Fructus Based on Separation and Quantification of Eight Active Ingredients and Chemometrics Analysis

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Abstract Coptidis rhizoma and Evodiae fructus could be compatible with different proportions, which composed of a series of prescriptions, including Zuojinwan (ZJW), Biantongwan (BTW), Ganlusan (GLS), Zhuyuwan (ZYW), and Fanzuojinwan (FZJW). Eight bioactive compounds, chlorogenic acid (1), jatrorrhizine (2), coptisine (3), palmatin (4), berberine (5), evodin (6), evodiamine (7), and rutaecarpine (8), were the main active ingredients in Coptidis rhizome - Evodiae fructus composition. In this paper, above 8 compounds were determined simultaneously in 105 batches of samples by high performance liquid chromatography tandem diode array detector (HPLC-DAD), in which 23 batches of BTW, 14 batches of ZYW, 23 batches of GLS, 20 batches of FZJW, 25 batches of ZJW, and 5 batches of ZJW preparations (ZJWps) were included. All calibration curves showed good linear regression ($r^2 > 0.9970$), good precision, and accuracy with overall intra and inter-day variations of 0.54-1.58% and 0.54-2.07%, respectively, and the overall recoveries of 98.05-100.86%. Subsequently, the principal component analysis (PCA) was used to evaluate and distinguish ZJW, GLS, ZYW, BTW, FZJW and ZJWps according to the contents of the detected 8 active ingredients. Through the PCA analysis, 5 kinds of prescriptions could be dispersed in different areas, and there was no intersection among them. Unexpectedly, 5 batches of ZJWps deviated from the scatters region belonged to ZJW, which indicated that there were some quality problems in ZJWps. This method could be applied to detect and distinguish Coptidis rhizome-Evodiae fructus prescriptions.

Keywords: Zuojinwan, Biantongwan, Zhuyuwan, Ganlusan, Fanzuojinwan, Coptidis rhizoma-Evodiae fructus, HPLC-DAD, chemometrics analysis

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1. Introduction

In clinical practice, Coptidis rhizoma has the effects of antiadipogenic activity [1], antibacterial, antioxidant, and cytotoxic activities [2], antitumor [3], antidiabetic [4], and Evodiae fructus possesses the actions of antinociceptive activity [5], antitumor [6,7], antibacterial [8]. Furthermore, various combinations of Coptidis rhizoma and Evodiae fructus had been used to treat gastro-intestinal disorders [9,10]. The two herbs are the main components of a series of similar formulas in Chinese herbal preparations, such as Zuojinwan (ZJW), Fanzuojinwan (FZJW), Biantongwan (BTW), Ganlusan (GLS), and Zhuyuwan (ZYW). Pharmacological studies showed that ZJW has the effects of inhibiting gastric secretion and pepsin, antiinflammatory, antifungal and analgesic [11], and had been widely

adopted for curing hypochondric and costal pain, stomach ache, acid regurgitation, nausea and upset in China [12].

Nowadays, it is widely accepted that multiple constituents are responsible for the mutual therapeutic effect. The main active ingredients in Coptidis rhizoma were a series of protoberberine alkaloids, including berberine, coptisine, palmatin, jatrorrhizine, and others [13,14,15]. In addition, the major active components in Evodiae fructus were evodin, chlorogenic acid, evodiamine, rutaecarpine, and other components. ZJW, GLS, ZYW, BTW, and FZJW were made from Coptidis rhizoma and Evodiae fructus in different proportions, as shown in Table 1. Therefore, it was necessary to establish a quality control method for the simultaneous determination of a variety of chemical constituents belonging to Coptidis rhizoma and Evodiae fructus.

Table 1. Ratio and dosage of ZJW, FZJW, ZYW, BTW, and GLS

Sample	Coptis:Evodia	Dosage (g)
Zuojin Wan (ZJW)	6:1	0.10
Fanzuojin Wan (FZJW)	1:6	0.10
Zhuyu Wan (ZYW)	3:2	0.10
Ganlu San (GLS)	2:1	0.10
Biantong Wan (GTW)	1:1	0.10
Zuojin Wan preparation (ZJWps)	6:1	0.10

Furthermore, according to the ratio of the amount of GLS, ZYW, and BTW, we hypothesized that the differences in the content of their main ingredients could not be significant. It was difficult to find out the slight changes in main composition of GLS, ZYW, and BTW. It was also difficult to distinguish ZJW, GLS, ZYW, BTW, and FZJW effectively according to the contents of their active ingredients change. Principal component analysis (PCA) is a very popular data exploration and reduction technique for extracting the most important information from the large, and confusing data sets [16,17,18]. The PCA was useful and powerful to evaluate and distinguish ZJW, GLS, ZYW, BTW, and FZJW according to the contents of the detected active ingredients.

In this study, 105 batches of samples were prepared, in which 23 batches of BTW, 14 batches of ZYW, 23 batches of GLS, 20 batches of FZJW, 25 batches of ZJW, and 5 batches of ZJW preparations (ZJWps) were included.

Figure 1. The chemical structures of 8 active compounds

1. Chlorogenic acid, 2. jatrorrhizine, 3. coptisine, 4. palmatin, 5. berberine, 6. evodin, 7. evodiamine, 8. rutaecarpine

Chlorogenic acid (1), jatrorrhizine (2), palmatin (4), berberine (5), evodin (6), evodiamine (7), rutaecarpine (8) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); Coptisine (3) was purchased from Prof. Hao Zhang (West China School of Pharmacy, Sichuan University, Chengdu, P.R. China). Their chemical structures were shown in Figure 1. HPLC-grade acetonitrile, methanol was purchased from Merck Company Inc. (Merck, Darmstadt, Germany). Ultra-pure water was prepared by a Milli-Q50 SP Reagent Water System (Millipore Corporation, MA, USA) for the preparation of samples and mobile phase solutions. Other reagents were of analytical grade.

2.2. HPLC-DAD Conditions

Agilent 1200 HPLC system (Agilent Corporation, USA) coupled with diode array detector (DAD) was used for determination. The chromatographic mobile phase was that 0.1% phosphoric acid and acetonitrile. The gradient program is presented in Table 2. Detection wavelength was set at 345 nm for compounds 1-5, and 210 nm for compounds 6-8.

Immediately, 8 active ingredients were detected simultaneously by HPLC-DAD. Finally, the PCA method was used to analyze the 105 batches of samples and distinguish ZJW, GLS, ZYW, BTW, and FZJW according to the contents results.

2. Materials and Methods

2.1. Reagents and Materials

Coptidis rhizoma was purchased from Nanjing Pharmacy (Nanjing, P.R. China), Evodiae fructus was purchased from Evodiae implantation depot (Hubei, P.R. China). The specific species of Coptidis rhizoma and Evodiae fructus were *Coptidis rhizoma* Franch and *Evodiarutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang, respectively, which were identified by Prof. Xiaobin Jia (Jiangsu Provincial Academy of Chinese Medicine, Nanjing, P.R. China). Zuojinwan, Fanzuojinwan, Biantongwan, Ganlusan, and Zhuyuwan were all self-made in our laboratory (Table 1). Five batches of ZJWps were purchased from Hubei Ruidesheng pharmaceutical limited company (Hubei, China).

Table 2. Solvent gradient program of HPLC-DAD analysis

Time (min)	A (0.1% H ₃ PO ₄)	B (HCN%)
0	95	5
7	92	8
13	89	11
25	75	25
45	70	30
50	70	30
70	40	60
80	95	5

2.3. Preparation of Standard Solutions and Sample Solutions

Mix standard stock solution containing chlorogenic acid (34.5 µg/mL), jatrorrhizine (35.0 µg/mL), coptisine (18.5 µg/mL), palmatine (12.9 µg/mL), berberine (42.6 µg/mL), evodin (35.3 µg/mL), evodiamine (41.8 µg/mL) and rutaecarpine (32.0 µg/mL) was prepared in methanol. The

solutions were stored in refrigerator at 4°C all the time. A set of standard solutions were prepared by the appropriate dilution of the stock solution with methanol.

0.1 g samples were all extracted with 50 mL, 50% methanol by sonicating for 30 min (200 W, 40 KHz), and then 50% methanol was added to compensate the lost volume. The supernatant solution was filtrated through a syringe filter (0.45 µm) and 20 µL were subjected to HPLC analysis.

2.4. PCA for SO Samples

The PCA was performed to analyze these samples by SPSS 16.0 software package (SPSS, Chicago, IL, USA). In this study, the contents of the 8 compounds were analyzed from 23 batches of BTW, 14 batches of ZYW, 23 batches of GLS, 20 batches of FZJW, 20 batches of ZJW, and 5 batches of ZJWps, which composed a data matrix with 105 rows and 8 columns for PCA analysis after normalization. The first three principal components (PCs) were extracted, and the scatter plot were obtained by plotting the scores of PC 1 versus PC 2 and PC 3.

3. Results and Discussion

3.1. Optimization of Chromatographic Condition

The optimization of chromatographic condition was guided by the requirement of obtaining better resolution of adjacent peaks. The optimal mobile phase, consisting of acetonitrile-0.1% phosphoric acid (H₃PO₄) in mobile phase, which leads to good resolution and satisfactory peak shape. Besides, it was observed that the separation could be affected by column temperature obviously, especially the protoberberine alkaloids in *Coptidis rhizoma*. When the column temperature was higher than 20°C, the peaks of protoberberine alkaloids were too broad to part. Therefore, the column temperature was set at 20°C during analysis (Figure 2).

Figure 2. Typical HPLC-DAD chromatograms of the standard solution, ZJW and ZJWps

A. Standard solution, B. ZJW, C. ZJWps

Peaks: 1. chlorogenic acid, 2. jatrorrhizine, 3. coptisine, 4. palmatin, 5. berberine, 6. evodin, 7. evodiamine, 8. rutaecarpine

3.2. Method Validation of HPLC-DAD

3.2.1. Linearity, LOD and LOQ

The stock solution of the eight standards was prepared and diluted to six appropriate concentrations for the establishment of calibration curves. The regression equations were achieved after linear regression of the peak areas versus the corresponding concentrations. The LOD and LOQ for each analyte under the chromatographic conditions were determined at the S/N of 3 and 10, respectively. The results of the regression indicated that all eight reference compounds showed good linearity in a relatively wide concentration range. The LOD and LOQ of the eight compounds were 0.41-2.77 ng and 1.36-9.93 ng, respectively (Table 3).

Table 3. Linear regression data, LOD and LOQ of eight standards

NO.	Compound	Wavelength	Regression equation	r ²	Linear range (µg)	LOD (ng)	LOQ (ng)
1	Chlorogenic acid	345nm	Y = 18.772 X - 8.8429	0.9992	0.0069-0.34	0.98	3.28
2	Jateorrhizine	345nm	Y = 51.967X + 57.592	0.9992	0.042-1.10	0.90	3.02
3	Coptisine	345nm	Y = 34.452 X + 10.966	0.9997	0.17-2.07	2.77	9.26
4	Palmatine	345nm	Y = 184.28X + 73.691	0.9996	0.058-1.05	0.91	3.03
5	Berberine	345nm	Y = 453.84X - 100.01	0.9999	0.28-3.99	1.28	4.27
6	Evodin	210nm	Y = 25.01X + 8.0808	0.9986	0.0019-1.06	1.29	9.93
7	Evodiamine	210nm	Y = 197.87X - 2.3609	0.9999	0.012-0.64	0.41	1.36
8	Rutacarpine	210nm	Y = 116.24X - 35.599	0.9970	0.0041-0.84	1.01	3.37

Y is the peak area in UV chromatograms monitored at 345 nm or 210nm, X is the concentration injected.

LOD refers to the limits of detection.

LOQ refers to the limits of quantification.

3.2.2. Precision, Repeatability, and Stability

Intra-day and inter-day variations were chosen to determine the precision of the developed assay. Three different concentrations of standards were prepared. The intra-day variation was determined by analyzing the six replicates within a single day. Inter-day variation was

examined in six consecutive days. Repeatability was confirmed with solutions prepared from BTW, and it was injected into the apparatus at 0, 2, 4, 8, 12, 16, and 24 h, respectively. Variations were expressed by RSD. All the results are summarized in Table 4. It indicated that the intra-day, inter-day, repeatability, and stability RSD values of the eight compounds were all less than 3.0%.

Table 4. Precision, repeatability and stability of eight compounds in sample (Biantongwan) (n=6)

Analytes	Precision			Repeatability (RSD%)	Stability (RSD%)
	Levels (µg/mL)	Intra-day RSD (%)	Inter-day RSD (%)		
1	1.38	0.94	0.97	0.60	1.10
	3.45	1.17	1.27		
	34.50	1.09	1.16		
2	1.40	1.53	1.89	1.26	1.54
	2.07	0.98	1.79		
	35.00	1.53	0.98		
3	0.74	0.70	1.65	1.58	2.07
	1.46	1.08	1.68		
	18.50	1.08	1.45		
4	0.52	1.03	1.14	0.54	0.61
	1.98	1.42	1.37		
	12.90	1.18	1.42		
5	1.70	1.80	2.31	1.02	1.75
	2.64	2.27	2.49		
	42.60	2.27	3.01		
6	1.41	1.43	1.48	1.48	1.76
	3.53	0.77	1.48		
	35.3	0.77	1.27		
7	1.28	0.64	0.76	0.76	0.61
	3.20	0.42	0.90		
	32.00	0.77	0.42		
8	1.67	1.12	1.15	0.58	0.54
	4.18	1.52	2.12		
	41.80	1.52	1.82		

3.2.3. Recovery

The recovery was performed by adding known amounts of the eight references. The spiked samples were then extracted, processed, and quantified in accordance with

the methods mentioned above. The overall recoveries lay between 98.05 and 100.86% for all reference compounds. The results were summarized in Table 5.

Table 5. Recovery of eight compounds in BTW and ZJWps (n=3)

Compounds	samples	Recovery		
		Amount added (mg)	Recovery (%)	RSD (%)
1	BTW	0.61	99.31	2.70
	ZJWps	0.15	99.79	1.47
2	BTW	1.98	99.87	1.64
	ZJWps	2.20	99.75	1.66
3	BTW	5.65	99.39	2.14
	ZJWps	4.51	98.71	1.18
4	BTW	2.02	99.76	2.29
	ZJWps	3.03	100.32	2.56
5	BTW	10.72	100.86	2.07
	ZJWps	24.26	98.05	1.43
6	BTW	1.98	100.48	1.08
	ZJWps	0.50	99.36	2.48
7	BTW	0.80	98.60	0.80
	ZJWps	0.17	99.73	2.00
8	BTW	0.31	99.38	1.76
	ZJWps	0.11	99.78	1.69

Table 6. Each content of eight active components in various samples (mg/g) (n=3)

Sample NO.	Prescription	Contents of analytes (mg/g, n = 3)								Total (mg/g)
		1	2	3	4	5	6	7	8	
1	BTW	2.36	8.07	22.79	8.67	41.43	7.95	3.04	1.17	95.49
2		2.06	7.77	19.76	8.11	36.54	7.34	2.58	1.22	85.38
3		2.11	7.57	21.32	7.87	43.33	7.25	2.78	1.43	93.66
4		2.21	6.32	23.54	8.54	48.34	7.93	3.22	1.43	101.54
5		1.98	8.23	24.14	6.79	36.47	6.58	3.54	1.27	89.00
6		2.15	8.11	18.74	8.09	34.40	7.32	3.65	1.22	83.69
7		1.92	7.45	19.73	7.43	42.66	6.52	2.44	1.45	89.60
8		1.95	7.35	21.65	8.32	41.66	7.43	3.44	1.07	92.87
9		2.17	8.42	20.65	7.98	41.22	6.99	2.43	1.43	91.29
10		2.24	8.04	17.44	8.22	33.44	6.95	2.54	1.54	80.41
11		2.43	8.01	23.88	8.82	36.30	7.22	2.88	1.52	91.06
12		2.41	7.67	21.38	8.65	37.55	7.45	3.12	1.42	89.66
13		2.08	8.46	16.99	9.32	38.40	7.22	3.54	1.74	87.75
14		2.28	8.72	22.24	5.64	28.55	8.35	3.48	1.53	80.79
15		2.16	7.83	18.65	8.32	26.45	8.45	3.44	1.65	76.96
16		1.95	6.54	24.54	8.67	37.41	6.97	3.37	1.57	91.02
17		1.97	7.94	23.35	9.54	40.33	8.22	3.11	0.99	95.45
18		1.96	7.68	18.99	9.64	45.62	7.37	2.67	0.96	94.89
19		2.09	7.88	19.54	8.44	39.97	7.87	2.89	1.07	89.75
20		2.34	8.18	21.06	8.61	46.43	7.66	3.32	1.19	98.79
21		2.21	8.86	22.42	6.86	40.09	8.95	1.78	1.65	92.82

22		2.51	8.07	23.76	7.18	33.14	7.43	3.23	1.56	86.87
23	BTW	2.08	7.33	22.45	6.50	38.73	6.60	2.34	2.00	88.02
24		1.95	9.43	28.32	8.43	34.45	7.22	2.43	1.33	93.56
25		2.54	8.22	26.65	7.98	44.32	6.83	2.76	1.43	100.73
26		3.64	7.60	21.74	7.18	54.32	6.77	2.95	2.01	106.21
27		2.43	8.23	27.54	10.54	58.34	8.43	1.54	1.62	118.68
28		2.53	7.72	28.32	7.62	30.33	5.75	1.43	1.37	85.07
29		1.79	7.45	28.54	5.94	26.53	5.99	1.31	2.03	79.58
30		2.83	7.19	29.43	6.26	26.12	4.81	1.55	1.43	79.63
31	ZYW	2.94	8.92	27.12	7.58	37.72	5.55	1.19	1.23	92.25
32		2.91	8.66	24.43	5.90	53.31	5.47	1.07	1.34	103.08
33		3.34	8.39	27.15	7.22	28.90	7.29	1.71	0.97	84.97
34		2.77	9.13	24.43	5.54	44.49	6.12	2.47	1.89	96.84
35		2.94	4.81	28.89	8.86	47.54	5.25	2.22	1.10	101.63
36		2.81	8.28	28.23	11.23	56.36	6.91	2.22	1.10	117.14
37		3.38	9.54	27.54	8.54	51.95	7.08	2.55	1.01	111.59
38		1.54	9.22	23.43	9.43	51.32	5.65	1.65	1.79	104.03
39		1.51	9.25	23.41	9.42	51.31	5.64	1.64	1.72	103.90
40		1.03	7.68	27.43	12.84	57.65	6.53	1.54	0.94	115.64
41		0.80	9.97	30.02	10.48	69.22	19.20	2.55	1.01	143.25
42	GLS	1.90	9.32	26.12	10.33	54.41	6.43	2.59	1.06	112.16
43		1.56	10.70	30.05	11.84	63.57	5.38	2.09	0.84	126.03
44		1.24	8.43	21.54	11.32	60.33	5.04	2.12	0.65	110.68
45		1.11	9.76	27.02	9.84	53.73	6.01	1.76	0.88	110.11
46		0.89	10.25	30.22	7.55	54.64	4.43	2.11	0.77	110.85
47		1.25	11.22	31.05	12.55	51.82	8.43	3.11	0.86	120.29
48		0.95	10.27	21.03	9.54	49.77	4.38	2.32	0.90	99.16
49		0.86	8.44	31.93	13.11	43.71	5.20	2.58	0.89	106.72
50		1.78	9.61	22.83	8.26	57.66	5.09	2.79	1.00	109.01
51		0.71	10.78	33.73	11.66	48.60	7.98	2.40	1.19	117.05
52		1.64	9.95	34.62	10.06	61.55	6.87	2.90	1.33	128.92
53	GLS	0.57	11.12	35.52	8.47	59.49	4.76	3.01	0.58	123.51
54		0.98	12.29	36.42	9.87	57.44	5.64	2.12	0.90	125.66
55		0.56	10.46	37.32	12.27	65.38	6.53	2.22	0.80	135.55
56		0.75	9.63	38.22	9.67	53.33	4.42	2.33	0.70	119.05
57		1.87	8.80	39.12	10.08	61.27	6.31	2.04	0.60	130.09
58		0.99	7.14	30.91	13.88	57.17	8.09	2.63	0.77	121.57
59		1.06	7.32	31.81	14.28	45.99	5.98	2.16	0.96	109.55
60		1.02	10.49	26.71	9.68	53.06	4.86	2.24	0.77	108.82
61		3.95	2.28	8.28	2.79	13.89	12.11	3.73	2.19	49.21
62		3.42	2.22	8.43	2.23	11.43	16.22	3.43	1.69	49.07
63		2.34	1.88	7.54	2.65	15.22	11.52	3.04	2.02	46.22
64		3.11	2.74	9.54	3.44	12.32	10.52	3.63	2.77	48.07
65	FZJW	2.43	2.32	9.17	2.42	11.22	10.23	2.73	3.13	43.65
66		2.05	2.47	8.43	1.78	14.28	9.65	3.76	1.87	44.29
67		1.71	2.53	8.76	2.19	12.90	8.33	3.34	2.63	42.39
68		2.48	2.53	7.14	2.68	15.86	10.39	3.45	2.23	46.75
69		3.54	2.65	8.66	1.98	11.81	16.44	2.73	1.83	49.65
70		3.71	1.71	7.60	2.87	11.77	15.50	2.54	2.93	48.62
71		1.28	1.77	7.81	2.77	9.72	14.55	3.23	3.03	44.16
72		4.13	2.43	9.02	1.97	7.68	12.61	4.82	3.38	46.02
73		4.98	1.89	6.73	2.56	10.63	9.66	2.76	2.23	41.43
74		4.83	2.95	8.44	3.46	13.59	11.71	2.96	3.33	51.25
75	FZJW	3.67	2.01	6.15	3.35	11.54	12.77	3.58	2.43	45.49
76		5.52	3.16	7.86	3.25	9.50	10.18	3.50	2.53	45.48
77		4.37	2.13	6.57	2.15	12.45	11.12	2.98	1.63	43.39
78		5.21	2.19	7.27	2.65	13.41	12.07	2.96	1.72	47.49
79		4.06	3.25	8.98	2.94	8.36	13.02	3.26	1.82	45.69
80		3.56	3.11	6.69	2.83	9.32	13.96	5.17	2.92	47.58
81		0.66	13.68	38.91	15.04	81.74	2.43	0.59	0.39	153.44
82		0.56	11.43	42.55	13.43	78.33	2.13	0.47	0.26	149.16
83		0.63	11.76	38.93	11.58	69.64	1.43	0.63	0.42	135.03
84		0.48	13.87	34.23	17.88	64.47	2.76	0.61	0.39	134.68
85		0.58	13.93	32.54	16.15	68.42	2.26	0.53	0.40	134.80
86		0.61	10.63	31.43	16.81	72.37	2.19	0.55	0.52	135.10
87	ZJW	0.55	9.54	29.01	17.76	86.31	3.32	0.67	0.33	147.50
88		0.44	15.43	36.91	12.15	90.26	2.54	0.49	0.35	158.57
89		0.73	12.51	34.80	14.81	74.21	2.67	0.41	0.46	140.61
90		0.92	10.50	42.70	19.48	88.16	2.40	0.53	0.38	165.07
91		0.81	15.49	40.59	12.15	72.11	1.73	0.45	0.29	143.63
92		0.40	14.49	38.49	10.81	96.06	2.44	0.68	0.36	163.72
93		0.59	17.48	46.38	12.28	88.58	3.48	0.80	0.27	169.87
94		0.68	14.48	34.28	11.74	80.03	1.73	0.52	0.23	143.69
95		0.77	16.47	42.17	17.21	71.47	3.18	0.48	0.39	152.16
96		0.76	18.46	40.07	16.68	82.92	2.06	0.49	0.35	161.80
97	ZJW	0.45	11.46	37.96	18.14	74.37	2.94	0.88	0.25	146.46
98		0.64	10.45	35.86	19.61	85.82	2.82	0.60	0.37	156.18
99		0.73	11.45	43.75	14.08	77.27	2.69	0.39	0.24	150.60
100		0.82	9.44	41.65	11.54	78.72	3.27	0.49	0.35	146.29
101	ZJWps	0.51	8.52	18.05	12.67	93.05	1.95	0.57	0.43	135.75

102	0.51	8.56	18.13	12.67	93.02	1.94	0.58	0.42	135.84
103	0.52	8.49	18.44	12.73	93.14	1.94	0.56	0.43	136.25
104	0.52	8.35	18.04	12.48	93.32	1.96	0.54	0.44	135.66
105	0.53	8.61	18.32	12.56	93.22	1.95	0.57	0.43	136.19

3.3. Quantitative Analysis of Samples

105 batches of samples were prepared, in which 23 batches of BTW, 14 batches of ZYW, 23 batches of GLS, 20 batches of FZJW, 25 batches of ZJW (including 5 batches of ZJWps) were included (Table 6). Based on the detected results of 105 batches of samples, the content ranges of the 8 components were chlorogenic acid 0.44-5.52 mg/g, jatrorrhizine 1.71-18.46 mg/g, coptisine 6.15-46.38 mg/g, palmatin 1.78-19.61 mg/g, berberine 7.67-96.06 mg/g, evodin 1.43-16.44 mg/g, evodiamine 0.39-4.82 mg/g, rutaecarpine 0.23-3.38 mg/g.

In 105 batches of samples, the contents of alkaloids in Coptidis rhizoma were significantly higher than the ingredients in Evodiae fructus. Meanwhile, ingredients contents of Coptidis rhizoma alkaloids were berberine > coptisine > palmatin > jatrorrhizine, and the content of berberine was the highest than other 7 compounds. With the increase of the amount of Evodiae fructus, the contents of chlorogenic acid, evodin, evodiamine and rutaecarpine

also increased in varying degrees. Meanwhile, evodin > chlorogenic acid > evodiamine > rutaecarpine, and the content of rutaecarpine was the lowest than other 7 compounds.

3.4. PCA of the Samples

To evaluate the variation of ZJW, FZJW, GLS, ZYW, and BTW, PCA was performed on the basis of the contents of 8 tested compounds from them. The first three principal components (PC 1, PC 2, and PC 3) with > 89.5% of the whole variance was extracted for analysis. Among them, PC 1 accounted for 79.742% of total variance, whereas PC 2 and PC 3 for 5.452% and 4.379%, respectively (Table 7). The remaining principal components were discarded owing to a minor effect on the model. According to their loadings, PC1 had good correlation with the 8 tested compounds. The above results suggested that most of the compounds might contribute to the classification of the samples.

Table 7. Total Variance Explained for principal component analysis

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	Variance%	Cumulative%	Total	Variance%	Cumulative%
1	6.378	79.726	79.726	6.378	79.726	79.726
2	0.437	5.457	85.183	0.437	5.457	85.183
3	0.350	4.373	89.556	0.350	4.373	89.556
4	0.243	3.032	92.588			
5	0.215	2.684	95.272			
6	0.159	1.991	97.263			
7	0.130	1.628	98.891			
8	0.089	1.109	100.000

Figure 3. The 3D scatter plots obtained by Principal component analysis (PCA) of 105 samples

A. FZJW, B. ZYW, C. BTW, D. GLS, E. ZJW, F. ZJWps

Through the PCA analysis, 5 prescriptions could be dispersed in different areas, and there was no intersection between them (Figure 3). Compared with ZYW and BTW, the scatters of ZJW, FZJW, GLS were scattered in a relatively wide areas. Interestingly, the FZJW and ZJW were respectively distributed in the uppermost and lowermost, which might be due to the huge difference of their drug proportions and 8 major chemical compositions. Similarly, ZYW, BTW, and GLS distributed relatively close, which was that the difference of drug proportions

and content of 8 compounds among them three were not significant.

Five batches of ZJWps overlapped together, which indicated that its production process and its homogeneity were relatively stable. However, 5 ZJWps did not distribute in the ZJW area, which deviated from the scatters region belonged to ZJW. Possible reasons for this phenomenon were as follows: (1) The Evodiae fructus used for the manufacture of ZJWps might be its processed products instead of crude ones, therefore the content of chlorogenic acid was lower than ZJW groups [19]. (2) Compared with ZJW groups, the contents of coptisine, palmatin, and jatrorrhizine were lower while only the content of berberine was higher, which demonstrated that the amount of required Coptidis rhizoma was reduced by taking the place of adding berberine directly. The main reason could be that the price of required Coptidis rhizoma herbs for ZJWps was higher than the corresponding berberine.

4. Conclusions

In present study, 8 active ingredients in 105 batches of the 5 prescriptions composed by Evodiae fructus and Coptidis rhizoma were analyzed by HPLC-DAD method. The assay had good performance in selectivity, recovery, precision and accuracy, and proved to be highly sensitive for their quality control. Differences among 5 prescriptions in the contents of 8 active ingredients had

been revealed by PCA. In Chinese Pharmacopoeia, the only indicator of the quality control of ZJW and ZJWps was the content of berberine [20], which did not fully reflect their true quality. This method can be applied to detect and distinguish these five prescriptions, which could be useful for quality control of ZJW, FZJW, GLS, ZYW, and BTW.

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