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Short communication

Simultaneous determination of six active components in crude and processed Fructus Corni by high performance liquid chromatography

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ABSTRACT

A simple and rapid HPLC method was established for simultaneously determining six active components in Fructus Corni. The six components were separated on an Agilent Zorbax Extend C_{18} column (250 mm × 4.6 mm, 5 μm) and detected by diode array detector (DAD). Mobile phase was composed of (A) aqueous phosphoric acid (0.1%, v/v) and (B) acetonitrile phosphoric acid (0.1%, v/v) using a gradient elution. Analyses were performed at 30 °C with a flow rate of 1.0 mL/min and UV detection at 218 nm, 240 nm and 284 nm. All calibration curves showed good linear regression ($r^2 \geq 0.9999$) within tested ranges. The LOD and LOQ were 0.11–1.69 μg/mL and 1.48–16.60 μg/mL, respectively. Overall intra-day and inter-day variations were less than 4.72%, and the average recoveries were 97.97–102.51% for the analytes. The developed method can be applied to the intrinsic quality control of Fructus Corni.

1. Introduction

Fructus Corni is derived from the dry ripe sarcocarp of *Cornus officinalis* Sieb. et Zucc. The crude drug or its processed products of jiu zheng pin (JZP) and qing zheng pin (QZP) are used clinically. JZP is produced after steaming the crude drug pre-steeped in wine, and QZP is produced after steaming the crude drug [1,2].

In the last decades, Fructus Corni has been extensively investigated in phytochemistry, and the results indicated that gallic acid, 5-hydroxymethylfurfural (5-HMF), morroniside, sweroside, loganin and cornuside were the main active components in Fructus Corni. Pharmacological studies on the components showed that they all had good biological activities. Gallic acid had anti-inflammatory activity [3] and bacteriostatic action [4,5]. Morroniside and loganin showed antioxidation and protective effects on rat mesangial cell proliferation [6]. Sweroside could resist D-aminogalactose hepatic injury [7]. Cornuside suppressed cytokine-induced proinflammatory, relaxated vasculature, and inhibited melanogenesis [8–10]. 5-HMF could improve blood circulation [11,12].

To our knowledge, previously reported analytical methods were developed to quantify only one or two types of components in Fructus Corni such as gallic acid [13–15], morroniside [16] and loganin [17–19]. In current study, an HPLC-DAD method was developed to quantify six components simultaneously in Fructus Corni.

2. Experimental

2.1. Samples, chemicals and reagents

Fructus Corni was collected from four suppliers (Henan, Shanxi, Zhejiang and Anhui in China). Gallic acid was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 5-HMF was purchased from Shanghai Yousi Biotechnology Co. Ltd. (Shanghai, China). Morroniside, sweroside and loganin were purchased from Institute of Jiangsu Pharmaceutical Research (Nanjing, China). Cornuside was prepared from our laboratory, and its identity was verified by LC–MS, ^{1}H NMR and ^{13}C NMR [20]. The purity for each standard compound was greater than 98% by HPLC analysis. The structures of these six compounds were shown in Fig. 1. All reagents with high grade were obtained from others.

2.2. HPLC analysis

Analyses were performed using HPLC system Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) with diode array detector. Detection wavelengths were set at 218 nm for gallic acid and cornuside, 240 nm for morroniside, sweroside and loganin, and 284 nm for 5-HMF. An Agilent Zorbax Extend C_{18} (250 mm × 4.6 mm, 5 μm)

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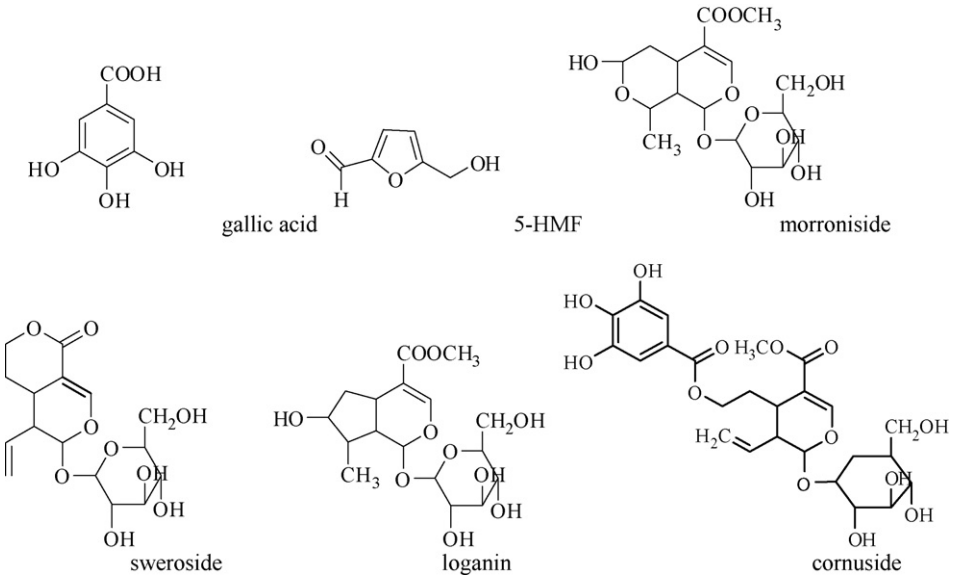

Fig. 1. The chemical structures of six active components in Fructus Corni.

was used with a flow rate of 1.0 mL/min. The injection volume was 10 μL and the column temperature was maintained at 30 °C. Mobile phase was composed of (A) aqueous phosphoric acid (0.1%, v/v) and (B) acetonitrile phosphoric acid (0.1%, v/v) using a gradient elution of 2% B at 0–10 min, 2–5% B at 10–15 min, 5–15% B at 15–45 min, 15–25% B at 45–55 min, 25–90% B at 55–70 min, 90% B at 80 min.

2.3. Preparation of standard solutions

Standard stock solution of gallic acid (0.428 mg/mL), 5-HMF (0.792 mg/mL), morroniside (1.660 mg/mL), sweroside (0.148 mg/mL), loganin (0.804 mg/mL) and cornuside (0.344 mg/mL) was prepared in 80% methanol. The standard stock solution was further diluted with 80% methanol to make 10 different concentrations including 1, 3/4, 1/2, 1/4, 1/5, 1/10, 3/40, 1/20, 1/40 and 1/100 of the original concentration. The solutions were filtered through a 0.45-μm membrane prior to injection.

2.4. Preparation of sample solutions

The powder of Fructus Corni samples, were precisely weighed (1.000 g), and transferred into dark brown calibrated flasks. They were extracted with 25 mL of 80% methanol in an ultrasonic bath for 45 min. Additional 80% methanol was added to make up the lost. The supernatants were filtered through a 0.45-μm membrane prior to injection.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Different mobile phase compositions were tested: (1) water–methanol; (2) water–acetonitrile; (3) aqueous phosphoric acid (0.1%, v/v)–acetonitrile phosphoric acid (0.1%, v/v); (4) aqueous ammonium acetate (0.5%, v/v)–acetonitrile. As a result, the combination of aqueous phosphoric acid (0.1%, v/v)–acetonitrile phosphoric acid (0.1%, v/v) for mobile phase was the best for

Table 1
Accuracy of the assay ($n = 6$)

Samples	Analytes	Original (mg ± S.D.)	Spiked (mg)	Determined (mg ± S.D.)	Recovery[a] (% ± S.D., R.S.D.%)
Crude drug	Gallic acid	0.67 ± 0.02	0.65	1.32 ± 0.01	100.13 ± 3.06, 3.06
	5-HMF	–[b]	2.20	2.18 ± 0.04	99.02 ± 1.83, 1.84
	Morroniside	15.28 ± 0.10	15.00	29.93 ± 0.51	97.65 ± 2.82, 2.89
	Sweroside	0.46 ± 0.02	0.45	0.92 ± 0.02	101.67 ± 2.48, 2.44
	Loganin	7.89 ± 0.07	7.80	15.60 ± 0.29	98.86 ± 3.40, 3.44
	Cornuside	6.26 ± 0.06	6.00	12.23 ± 0.12	99.50 ± 2.01, 2.02
JZP	Gallic acid	2.95 ± 0.10	2.95	5.84 ± 0.15	97.97 ± 4.71, 4.81
	5-HMF	4.96 ± 0.08	4.90	9.87 ± 0.17	100.20 ± 2.95, 2.94
	Morroniside	13.95 ± 0.10	13.80	27.64 ± 0.27	99.19 ± 2.54, 2.56
	Sweroside	0.55 ± 0.02	0.55	1.10 ± 0.03	99.70 ± 3.34, 3.35
	Loganin	7.04 ± 0.10	7.00	14.02 ± 0.13	102.51 ± 2.43, 2.37
	Cornuside	5.30 ± 0.09	5.00	10.23 ± 0.15	98.70 ± 3.34, 3.38
QZP	Gallic acid	3.28 ± 0.02	3.25	6.51 ± 0.06	99.18 ± 1.97, 1.99
	5-HMF	8.45 ± 0.03	8.45	16.89 ± 0.26	99.88 ± 3.34, 3.35
	Morroniside	11.70 ± 0.02	11.65	23.21 ± 0.25	98.81 ± 2.06, 2.08
	Sweroside	0.67 ± 0.02	0.70	1.38 ± 0.03	100.95 ± 2.81, 2.78
	Loganin	7.50 ± 0.01	7.50	14.96 ± 0.10	99.47 ± 1.40, 1.40
	Cornuside	4.62 ± 0.13	4.50	9.18 ± 0.22	101.44 ± 3.37, 3.32

[a] Recovery (%) = $(\text{amount}_{\text{determined}} - \text{amount}_{\text{original}})/\text{amount}_{\text{spiked}} \times 100$.
[b] Not detected.

Table 2
Contents of the six components in Fructus Corni (mg/g ± S.D., $n = 5$)

Samples	Suppliers	Gallic acid	5-HMF	Morroniside	Sweroside	Loganin	Cornuside
Crude drug	Henan	0.81 ± 0.03	–[a]	15.46 ± 0.59	0.63 ± 0.03	8.28 ± 0.11	6.28 ± 0.27
	Shanxi	0.75 ± 0.03	–	17.07 ± 0.70	0.60 ± 0.02	6.04 ± 0.14	5.63 ± 0.14
	Zhejiang	0.74 ± 0.03	–	13.05 ± 0.43	0.59 ± 0.02	7.64 ± 0.17	5.53 ± 0.25
	Anhui	0.73 ± 0.03	–	12.58 ± 0.50	0.75 ± 0.03	8.11 ± 0.30	4.91 ± 0.19
JZP	Henan	3.06 ± 0.07	3.17 ± 0.14	13.66 ± 0.62	0.71 ± 0.02	7.66 ± 0.34	5.15 ± 0.34
	Shanxi	2.76 ± 0.09	3.07 ± 0.10	11.61 ± 0.41	0.64 ± 0.03	5.75 ± 0.22	4.28 ± 0.15
	Zhejiang	2.95 ± 0.11	3.57 ± 0.15	11.36 ± 0.22	0.82 ± 0.02	7.31 ± 0.20	4.89 ± 0.08
	Anhui	3.39 ± 0.14	3.00 ± 0.13	12.00 ± 0.19	0.92 ± 0.03	7.79 ± 0.27	4.36 ± 0.11
QZP	Henan	3.16 ± 0.09	5.62 ± 0.13	11.21 ± 0.24	0.94 ± 0.03	6.43 ± 0.22	4.32 ± 0.15
	Shanxi	3.15 ± 0.10	6.45 ± 0.17	10.86 ± 0.41	0.83 ± 0.02	5.56 ± 0.11	4.67 ± 0.05
	Zhejiang	2.85 ± 0.05	5.81 ± 0.10	9.57 ± 0.37	0.75 ± 0.02	4.93 ± 0.09	4.25 ± 0.05
	Anhui	4.69 ± 0.12	6.35 ± 0.27	10.26 ± 0.32	1.40 ± 0.05	6.93 ± 0.29	4.31 ± 0.18

[a] Not detected.

separation. Furthermore, other chromatographic variables were also optimized, including analytical columns (Hanbon Hedera ODS-2, Hanbon Lichrospher C_{18} and Agilent Zorbax Extend C_{18}), the column temperatures (20 °C, 25 °C and 30 °C) and the flow rates (0.8 mL/min and 1.0 mL/min). Eventually, the optimal separation was achieved on an Agilent Zorbax Extend C_{18} column (250 mm × 4.6 mm, 5 μm) at a column temperature of 30 °C with a flow rate of 1.0 mL/min.

3.2. Calibration curves, limits of detection and quantification

The calibration curves were performed with 10 different concentrations in triplicate. The regression equations were calculated in the form of $y = ax + b$, where y and x were peak area and concentration. The LOD was determined at a signal-to-noise ratio of 3, and the LOQ was determined as the lowest concentration in the linear range of each analyte.

The regression equations (linear ranges) were $y = 71.145x + 24.391$ (4.28–428.00 μg/mL, gallic acid), $y = 67.844x + 216.71$ (7.92–792.00 μg/mL, 5-HMF), $y = 13.934x + 12.33$ (16.60–1660.00 μg/mL, morroniside), $y = 19.59x - 1.7924$ (1.48–148.00 μg/mL, sweroside), $y = 15.357x + 1.3993$ (8.04–804.00 μg/mL, loganin), $y = 9.1877x - 1.4408$ (3.44–344.00 μg/mL, cornuside). All the marker substances showed good linearity ($r^2 \geq 0.9999$). The LOD and LOQ of the six analytes were 0.11–1.69 μg/mL and 1.48–16.60 μg/mL, respectively.

3.3. Precision, repeatability and stability

Intra-day and inter-day variations were chosen to determine the precision of the developed assay by analyzing 1/5 dilution of the standard stock solution. The intra-day variation was determined by analyzing the six replicates within 1 day. Inter-day variation was examined in six consecutive days. Overall intra-day and inter-day variations were less than 4.72%.

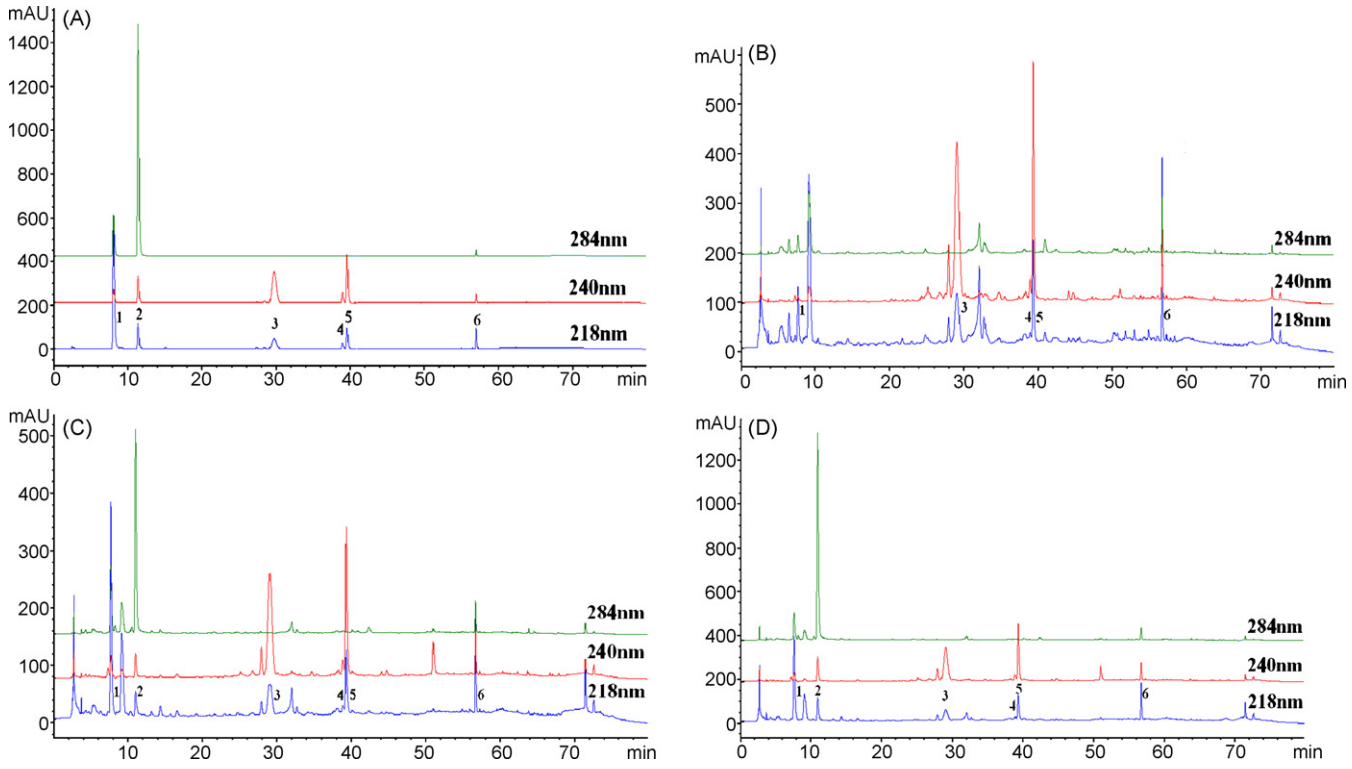

Fig. 2. Typical chromatograms of reference compounds (A), crude drug from Henan (B), JZP from Henan (C), QZP from Henan (D) in different detection wavelengths. (1) Gallic acid; (2) 5-HMF; (3) morroniside; (4) sweroside; (5) loganin and (6) cornuside.

To further evaluate the repeatability of the developed assay, Fructus Corni was analyzed in six replicates as described above. The contents of six compounds in Fructus Corni were calculated from the corresponding calibration curves. The relative standard deviations (R.S.D.s) were taken as measurements of repeatability. Stability was tested with Fructus Corni at room temperature and analyzed at 0 h, 2 h, 4 h, 8 h, 12 h, 24 and 48 h within 2 days, respectively. The R.S.D.s of repeatability test and stability were not more than 4.01% for all analytes.

3.4. Accuracy

Accuracy was determined by the recovery test. An appropriate amount of Fructus Corni powder was weighed and spiked with known amount of each standard compound. They were then treated and analyzed as described above. Each sample was analyzed in six replicates. The total amount of each analyte was calculated from the corresponding calibration curve. The results of recovery test were shown in Table 1.

3.5. Sample analysis

The contents of six compounds in Fructus Corni samples analyzed were listed in Table 2. The HPLC chromatograms of standards and samples were shown in Fig. 2.

The results showed that the content of each compound in crude drug, JZP and QZP varied significantly. For instance, for Fructus Corni collected from Henan, the content of gallic acid was lower in crude drug (0.81 mg/g), but higher in JZP (3.06 mg/g) and QZP (3.16 mg/g), 5-HMF was hardly detected in crude drug, with lower content in JZP (3.17 mg/g), but higher in QZP (5.62 mg/g), the contents of morroniside, loganin and cornuside in crude drug (15.46 mg/g, 8.28 mg/g and 6.28 mg/g) were higher than those in JZP (13.66 mg/g, 7.66 mg/g and 5.15 mg/g) and QZP (11.21 mg/g, 6.43 mg/g and 4.32 mg/g), the content of sweroside in crude drug (0.63 mg/g) was lower than those in JZP (0.71 mg/g) and QZP (0.94 mg/g). The variations might result from different processing procedures for Fructus Corni. It was reported that processing or heating could drastically increase the content of 5-HMF [21] and tannin in Fructus Corni was hydrolyzed to generate gallic acid owing to high temperature [22].

It could also be seen that the total contents of six compounds varied slightly in the same type of samples from different suppliers, which might be due to the differences in soils and climates in each region. Thus it is necessary to control the main active components in Fructus Corni by good agricultural practice (GAP) and the norm of Chinese medicinal materials processing.

4. Conclusion

An HPLC-DAD method has been developed to simultaneously determine six active components in Fructus Corni. This newly established method is validated as simple, precise and accurate. It can be used as a valid analytical method for intrinsic quality control of Fructus Corni.

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