Determination of organic acids for quality evaluation in *Coptis* herbs by ion chromatography

Dongmei Li¹ · Lili Zhou² · Qingwei Wang³ · Yang He¹

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Abstract

Coptis herbs are important herbal medicinal materials. The bioactive composition, the quality and medicinal efficacy of these herbs, are determined significantly by their geo-authentic features. Among the effective components of these herbs are seven organic acids (quinic, acetic, formic, tartaric, malic, succinic, and oxalic acids). However, no quantitative data of these seven acids in these herbs are available. Therefore, we developed a method for simultaneous separation and determination of the seven organic acids in *Coptis* herbs using gradient ion chromatography (mobile phase and gradient were shown in Table 1). The seven acids were separated and determined in no more than 35 min. We found that the organic acid levels in *C. teeta* was obviously higher than in *C. chinensis* and *C. deltoidea*, in particular, the content of quinic acid in *C. teeta* was about eight times than that in *C. chinensis* and *C. deltoidea*. Furthermore, we analyzed the relationships between the contents of organic acids and clinical effects, and found that organic acids (content of total acids or content of quinic acid) could act as a reference ingredient for quality evaluation in *Coptis* herbs. Our studies would lay the foundation for effective quality evaluation of these herbs.

Keywords *Coptis* herbs · Organic acids · Ion chromatography · Herbal quality

Introduction

Coptis herbs are important herbal medicinal materials. They are the dried rhizomes of ranunculaceous plants including (1) *Coptis chinensis* Franch., (2) *Coptis deltoidea* C.Y. Cheng et Hsiao and (3) *Coptis teeta* Wall (Qiao et al. 2009; Chen et al. 2017). All these three species grow in Southwest China (Ohura et al. 2012; He et al. 2017). Many clinical studies have shown *Coptis* herbs have antipyrexial effects, along with broad-spectrum antibacterial, antioxidant and anti-tumor activity. They have been used to treat diarrhea, dysentery, jaundice, hematemesis, carbuncles, and abscesses

(The pharmacopoeia of the People's Republic of China 2015).

It is known that the bioactive constituency, medicinal quality and efficacy of Chinese herbs are determined by their geo-authentic features (Chen et al. 2011; Woo et al. 1999; Liang et al. 2013). Moreover, compared with other herbs, high-quality of herbs are better in clinical effects. For *Coptis* herbs, inspection of their morphological markers is a traditional means for species classification or taxonomy (Vandebroek 2006). However, as these markers are unstable, their use in classifying different species or in evaluating the quality of the herb is challengable. Therefore, it is necessary to find more efficient alternatives to assist with quality control of dry *Coptis* herbs (Cheng et al. 1997).

Organic acids are the main active constituents of *Coptis* herbs and are suitable marker compounds (Deyl 1998). Most organic acids are soluble in water and are stable under typical processing conditions that utilize organic solvents, ion exchangers and alkaline solutions. There are a variety of organic acids in *Coptis* herbs, including vanillic, ferulic, quinic, acetic, formic, tartaric, malic, succinic acid and so on (Li et al. 2012). They have undergone little characterization

Dongmei Li and Lili Zhou contributed equally to this work.

✉ Yang He
hawkheyang@163.com

¹ College of Medical Technology, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

² National Institute of Measurement and Testing Technology, Chengdu 610021, China

³ Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

Table 1 KOH gradient for simultaneous separation of organic acids on AS11-HC columns

<i>T</i> (min)	<i>C</i> (KOH) (mmol/L)
0–8	1
8–18	1–15
18–28	15–30
28–38	30–60
38–45	1

with *Coptis* herbs, and their contents in *Coptis* herbs, indeed, have not been reported yet.

Nowadays, organic acids are mainly detected in Chinese medicinal materials via thin layer chromatography, capillary electrophoresis, high performance liquid chromatography and ion chromatography (IC) (Zhu et al. 2012; Jayaprakash and Sakariah 1998; Yang et al. 2005). Especially, IC is very suitable for organic acid detection. It can separate and detect ionic species in liquid samples and has advantages of simple operation with short analysis time (Fischer 2002; Cinquina et al. 2004), as well as having high sensitivity and a low detection limit. Thus, in this study, we developed an IC method to simultaneously determine the contents of seven organic acids (quinic, acetic, formic, tartaric, malic, succinic and oxalic acid) in *Coptis* herbs. With these data, we analyzed the relationships between the organic acid contents and the herbal qualities of *Coptis* herbs from three different botanical origins. This study provides a method for the determination of organic acid contents and for quality evaluation of *Coptis* herbs.

Materials and methods

Sample collection

Coptis herbs from three different botanical origins were collected from different habitats (*C. chinensis* from Shizhu, Chongqing City; *C. teeta* from Fugong, Yunnan Province; *C. deltoidea* from Hongya, Sichuan Province). Voucher specimens were deposited at Chengdu University of Traditional Chinese Medicine, Chengdu, China.

Sample preparation

Coptis herbs samples were ground then 2 ± 0.002 g was weighted and placed in a 250 mL volumetric flask along with 100 mL of ultra-pure water. The samples were mixed well before ultrasonic extraction for 30 min. The supernatant was filtered through a disposable 0.2 µm membrane filter (Membrana, Germany) and a RP cartridge by turns. RP cartridge was first washed with 5 mL methanol and then with 10 mL deionised water. About 5 mL of the sample solution

were loaded on the cartridge and allowed to flow at the rate of 1 mL/min. The first 3 mL elutes were rejected, and the following 2 mL were collected for direct injection into the ion chromatograph.

IC conditions

The IC was undertaken with a Dionex Corporation (Sunnyvale, CA, USA) ICS-2100 system and IonPac AS11-HC anion analytical column (250 mm × 4 mm). A gradient elution was performed with a mobile phase of aqueous potassium hydroxide solution at a flow rate of 1.0 mL/min. The temperature of the column was 30 °C and the detector temperature was 35 °C. Injection volume was 25 µL. The gradient program employed is given in Table 1.

Standard curve

Fifty mg of each organic acid standard (quinic, acetic, formic, tartaric, malic, succinic and oxalic acid), which were brought from Sigma–Aldrich (Shanghai) Trading Co. Ltd. were dissolved in ultra-pure water to a total volume of 50 mL and stored at 4 °C. The mass concentration of each organic acid was 1000 µg/mL. Standard solution in volumes of 0.5, 1.0, 2.0, 5.0 and 10.0 mL were added into a 25 mL volumetric flask and calibrated to the scale line with ultra-pure water, and stored at 4 °C after mixing well. The working solution for the standard curve was injected into the IC, and the corresponding peak area was measured. The corresponding standard curve was drawn with the standard working fluid concentration as the abscissa and the peak area as the vertical axis.

Data analysis

The organic acids in the three kinds of *Coptis* herbs were determined by IC. Each 25 µL sample was injected following the above method. The experiments were repeated for three times, and the results were represented by their mean ± standard error.

Results and discussion

Making of standard curves

Standard curves of each of the organic acids were successfully plotted and showed good linear correlation (Table 2). Malic and succinic acid overlapped and co-eluted so were combined for analysis with good linearity ($r = 0.99582$). Quinic acid had the best linear relationship ($r = 0.99998$). Overall, the standard curves for all of the target organic acids

Table 2 Linear regression and linearity for organic acids analyzed

Organic acids	Linear regression	Linearity	Linear range (µg/mL)	Area (µS*min)	Retention time (min)
Quinic acid	$y=0.0267x+0.0003$	0.99998	0.5–100	0.284	5.50
Acetic acid	$y=0.0498x+0.0311$	0.99678	0.5–25	0.706	6.98
Malic acid	$y=0.0434x+0.0708$	0.99582	0.5–25	1.005	19.52
Succinic acid					
Tartaric acid	$y=0.0617x+0.0500$	0.99919	0.5–25	0.926	20.13
Oxalic acid	$y=0.0830x+0.0286$	0.99937	0.5–25	0.996	22.25
Citric acid	$y=0.0451x+0.0246$	0.99771	0.5–25	0.469	29.95

Table 3 Limits of detection and quantification for organic acids analyzed

Organic acids	Detection limit		Quantification limit	
	Concentration (mg/L)	Content (µg/g)	Concentration (mg/L)	Content (µg/g)
Quinic acid	0.19	9.6	0.64	32.1
Acetic acid	0.07	3.7	0.25	12.4
Malic acid	0.23	11.5	0.77	38.5
Succinic acid				
Tartaric acid	0.75	37.5	2.50	125.0
Oxalic acid	0.83	41.7	2.78	138.9
Citric acid	0.44	22.1	1.47	73.5

met experimental requirements and could be used for our experiments.

Relative standard deviation and recovery

Limits of detection and quantification are two fundamental elements of method validation. The detection limit for each of the organic acids was three times the signal-to-noise ratio, and the limit of quantitation was ten times the signal-to-noise ratio. The detection and quantitative limits (as detection limit/quantitative limit) were 0.19/0.64, 0.07/0.25, 0.23/0.77, 0.75/2.50, 0.83/2.78 and 0.44/1.47 mg/L for quinic, acetic, malic + succinic, tartaric, oxalic, and citric acid, respectively (Table 3), with acetic acid having the lowest values (0.07/0.25 mg/L).

Using the above method, we took *C. teeta* as an example to analyze the relative standard deviation (RSD) and recovery. The RSD of each organic acid was less than 10%, which indicated that this method was reproducible. Add three levels of each organic acid standard to the a *C. teeta* sample (add 50, 100, 150% of the organic acid concentration in the sample). The recovery rates of all targets were between 70.1 and 117.4% (Table 4). Acetic acid was the lowest (70.1–79.4%) and tartaric acid was the highest (112.7–117.4%). These results indicated that this method can be used for quantitative analysis of organic acids in *Coptis* herbs.

Table 4 Relative standard deviations of reproducibility and recovery of organic acids analyzed

Organic acids	Relative standard deviation (%)	Recovery (%)
Quinic acid	3.09	87.1–96.8
Acetic acid	6.29	70.1–79.4
Malic acid	3.09	82.8–98.1
Succinic acid		
Tartaric acid	7.89	112.7–117.4
Oxalic acid	4.43	86.1–93.4
Citric acid	7.64	81.0–95.8

Analysis of organic acid contents in *Coptis* herb samples

Typical chromatograms of standards organic acids and three *Coptis* herbs samples (*C. chinensis*, *C. deltoidea* and *C. teeta*) are shown in Fig. 1, and the unassigned peaks were inorganic anions, for example, F^- , SO_4^{2-} , NO_3^- , Cl^- and PO_4^{3-} . The retention times of all organic acids were less than 35 min.

Total organic acid content was highest in *C. teeta* (45 mg/g), which was about three times higher than in *C. chinensis* and *C. deltoidea* (Table 5). Specifically, the content of quinic acid, and malic and succinic acid combined were highest in *C. teeta* (27.83 and 15.25 mg/g, respectively) comprising more than 75% of the total organic acid content and reaching up to 94%. The content of each organic acid in *C. teeta* was quite different from the other two *Coptis* herbs (Fig. 2). This method is the first to describe the detection of organic acid content in *Coptis* herbs. It can detect seven organic acids rapidly and simply, which has important application value.

Relationships between organic acids and drug quality

In the past, research on the active ingredients of traditional Chinese medicines has focused primarily on flavonoids, saponins and alkaloids, while the physical activity and function

Fig. 1 Representative ion chromatograms of: **a** standards organic acids; **b** *C. deltoidea*; **c** *C. teeta*; **d** *C. chinensis*. 1 quinic acid, 2 acetic acid, 3 succinic acid + tartaric acid, 4 tartaric acid, 5 oxalic acid, 6 citric acid. The unassigned peaks were inorganic anions

Table 5 Organic acids concentrations of three different *Coptis* herbs

Organic acids	<i>C. deltoidea</i> (mg/g)	<i>C. teeta</i> (mg/g)	<i>C. chinensis</i> (mg/g)
Quinic acid	4.00 ± 0.15	27.83 ± 1.33	2.90 ± 0.13
Acetic acid	1.03 ± 0.03	0.18 ± 0.01	0.78 ± 0.04
Malic acid	9.93 ± 0.46	15.25 ± 0.69	9.82 ± 0.43
Succinic acid			
Tartaric acid	0.76 ± 0.03	0.46 ± 0.02	0.96 ± 0.04
Oxalic acid	1.25 ± 0.06	0.34 ± 0.01	0.64 ± 0.03
Citric acid	1.40 ± 0.07	1.53 ± 0.06	1.19 ± 0.05

of organic acids have not been taken seriously (Cushnie and Lamb 2005; Chen et al. 2008). Later studies found that organic acids had pharmacological effects against inflammation (Dharmappa et al. 2008; Takada et al. 2010), oxidation (Wu et al. 2008), platelet aggregation (Punithavathi et al. 2011) and thrombosis (Miura et al. 2010), and an important role in the treatment of cardiovascular diseases such as myocardial ischemia, atherosclerosis and hypertension. *Coptis* herbs have also been shown to have antiviral and antibacterial activity, and to reduce blood pressure. Hence, organic acids are likely to be the main active constituent of *Coptis* herbs, and can be used as an indicator to evaluate the quality of medicinal materials.

Many studies have also shown that the organic acid composition and content can affect the quality of Chinese herbal medicines. Zhang et al. (2007) reported that organic acid content directly affected the quality of honeysuckle. However, for *Coptis* herbs, no study has been published about the relationships between organic acid content and herbal qualities. In this research, we found that the content of organic acids in *C. teeta* was obviously different in *C. chinensis* and *C. deltoidea* (Fig. 2; Table 5). In particular, the content of quinic acid in *C. teeta* was about eight times that in *C. chinensis* and *C. deltoidea*. In the effect of medicine, *C. teeta*

was also obviously superior to *C. chinensis* and *C. deltoidea*. Some researchers have previously reported that *C. teeta* is better quality than the other variants (Zhao et al. 2001). Certainly, based on the content of organic acids, our research suggests *C. teeta* has higher quality than *C. chinensis* and *C. deltoidea*. In this regard, the content of organic acids can be used to distinguish these three different medicinal herbs. Thus, our results suggest that organic acids can act as an active ingredient for auxiliary identification of the qualities of *Coptis* herbs. This can assist with identification of medicinal materials.

Conclusion and perspectives

In this study, a new gradient ion chromatography method was developed for separation and determination of seven organic acids (quinic, acetic, formic, tartaric, malic, succinic and oxalic acid) in *Coptis* herbs. The organic acid contents of three different *Coptis* herbs (*C. chinensis*, *C. teeta* and *C. deltoidea*) were determined, and found to be higher in *C. teeta* than in *C. chinensis* and *C. deltoidea*. The relationships between the organic acid content and herbal quality indicated that organic acids can act as an active ingredient for auxiliary identification of the herbal qualities of *Coptis* herbs.

Fig. 2 The percentage of seven organic acids in three *Coptis* herbs samples

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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