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# Sensitive analysis and simultaneous assessment of pharmacokinetic properties of crocin and crocetin after oral administration in rats

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ABSTRACT

Crocin and crocetin in rat plasma were simultaneously analysed using ultra-performance liquid chromatography tandem mass spectroscopy (UPLC–MS/MS), and method was fully validated. For the first time, levels of both crocin and crocetin in plasma were profiled after oral administration of crocin, and this UPLC–MS/MS approach was applied to evaluate pharmacokinetics and relative bioavailability of crocin and crocetin in rats. It was shown that crocin transformed into crocetin quickly in the gastrointestinal tract, and crocetin was 56–81 fold higher exposed in rat plasma than crocin after oral administration of crocin. A comparison study revealed that an oral administration of equal molar crocin achieved higher exposure of crocetin in rat plasma than that of crocetin. It was suggested that oral administration of crocin has the advantages over crocetin, and crocetin may be the active component potentially responsible for the pharmacological effect of crocin.



## 1. Introduction

Saffron is being used primarily as a spice and in many specialty dishes for coloring and flavoring food preparations, as well as medication in folk medicine [1,2]. The main compounds of the coloring pigment are crocin and its aglycone, crocetin [3]. They belong to members of natural carotenoids families and crocin is known as rare natural water-soluble yellow pigments [4]. In addition, crocin and crocetin are also found in the fruits of *Gardenia jasminoides* Ellis which is a popular ornamental shrub grown worldwide.

The pleiotropic health-promoting effects of crocin have been reported extensively in pre-clinical investigation. Previous studies have demonstrated that crocin could be used for the treatment of cardiovascular diseases due to its potential antihyperlipidemic effects [5], inhibition of calcium channels [6] and pronounced antioxidant reactivity [7–9]. Advanced pharmacological studies also have verified its brain protection effect in cerebral ischemia [10,11] and antitumor effects [12]. Similar to crocin, crocetin possesses various biological activities such as antioxidant [13], anti-inflammatory [14,15], anti-atherosclerotic [16,17], insulin resistance improvement [18,19] and neuroprotection [20].

Despite of the intensive interests on biological and pharmacological activities, a few studies focused on the pharmacokinetics of crocin and crocetin. Due to the concentration of crocin was very low in vivo, the methods reported in previous studies were not sensitive enough to meet the quantitative demands of crocin in biological samples, and only its metabolite, crocetin could be detected and profiled [21–23]. Hence, this study aimed to develop a specific and sensitive method to assess crocin and crocetin simultaneously in biological samples, and apply this method to evaluate pharmacokinetic properties of crocin and crocetin in rats.

To the best of our knowledge, this is the first report on comparative assessment of pharmacokinetic properties of crocin and crocetin, and simultaneous profile of crocin and crocetin to evaluate pharmacokinetic properties of crocin after its oral administration.

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## 2. Materials and methods

### 2.1. Animals

A total of 64 Sprague-Dawley rats (200 ± 20 g, half male and half female) were purchased (Shanghai Super – B&K Laboratory Animal Center, Shanghai, China), and housed in cages separately in clean grade SPF, with free access to chaws and water for 7 days before experimentation. After fastening overnight the rats were given with coocetin or crocin as described in "Pharmacokinietic study". The animial study and the protocols were approved by the Animal Ethics Committee of China Pharmaceutical University.

### 2.2. Materials

Crocin (also named crocin-I, $C_{44}H_{64}O_{24}$, molecular weight: 976.96, purity: 99.33%) and crocetin ($C_{20}H_{24}O_4$, molecular weight: 328.4, purity: 96.61%) were purchased from Chengdu Biopurify Phytochemicals Ltd (Chengdu, China) (Fig. 1). Digoxin was used as the internal standard (IS, purchased from Jiangsu Institute for Food and Drug Control, Nanjing, China). Acetonitrile was of HPLC-grade (purchased from Merck, Germany). Deionized water was obtained in a Milli-Q ultra-pure grade water system (Millipore, Bedford, USA), with a minimum resistance of 18.2 MΩ. The other chemicals and solvents were routinely available in our lab (analytical grade).

### 2.3. Preparation of stock solutions, calibration standards and control samples

An aliquot of 10.0 mg crocin was accurately weighed, dissolved in 3 mL water, then diluted with methanol to a final volume of 10 mL. While 10.0 mg crocetin was accurately weighed and dissolved in 10 mL pyridine (stored at 4 °C). Digoxin was also accurately weighted and diluted in methanol to 2.0 μg/mL. The primary stock solutions were diluted appropriately in methanol, and then diluted with blank rat plasma to produce the samples at a series concentrations of 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL, for crocin and crocetin, respectively.

### 2.4. Sample preparation

The plasma samples were thawed at 37 °C for 30 min, and then extracted using a liquid–liquid extraction method was used to extract the plasma samples. In detail, an aliquot of 45 μL plasma was added with 5 μL IS (2.0 μg/mL) and 1.0 mL of water-saturated *n*-butanol. After the mixtures were vigorously vortex-extracted for 10 min, they were centrifuged at 8000 × *g* for 5 min. An aliquot of 0.8 mL supernatant was transferred into an tube and evaporated to dryness in a rotary evaporator (ThermoFisher Savant SpeedVac Conncentrator System, USA). Finally, the dried residue was reconstituted with 100 μL of acetonitrile-water (v/v = 6:4), and 5 μL was injected into the UPLC–MS/MS for analysis.

### 2.5. Instrument and parameters

To achieve a sensitive and reproducible determination of the analytes, the UPLC–MS/MS-8050 (Shimadzu, Japan) system was employed. The system includes LC-30A binary pump, autosampler, electrospray ionization source, and 8050 triple quadrupole mass spectrometer. The system was controlled and the data was acquired using the software Ver.5.6 of LabSolutions (Shimadzu, Japan).

Chromatographic separation of the analytes was performed on a C18 column (100 mm × 2.1 mm, 3 μm, Atlantis® T3, Waters). The aqueous mobile phase (solvent A) contained 0.1% formic acid, while the organic mobile phase (solvent B) was acetonitrile. The mobile phase was programed in abinary gradient elution (flow rate at 0.2 mL/min) as follows: 0–1.0 min (10% B), 1.0–2.0 min (10–65% B), 2.0–4.5 min (65% B), 4.5–6.0 min (65–75% B), 6.0–6.3 min (75–95% B), 6.3–8.0 min (95% B), 8.0–8.8 min (95–40% B), 8.8–10.5 min (40% B), 10.5–11.0 min (40–10% B), 11.0–13.0 min (10% B).

The negative mode was selected and the analytes was quantitatively monitored using multiple reaction monitoring (MRM). The MRM transitions were *m/z* 975.45 → 651.20 for crocin, and *m/z* 327.40 → 239.30 for crocetin, and *m/z* 779.50 → 649.40 for digoxin (IS). The ion spray voltage was optimized and maintained at 4000 V. For efficient ionization of the analytes, desolventizing line was set at 250 °C, while the temperature of ion source and heat block were set at 350 °C. The values of collision energy were set at 25, 14 and 36 eV for crocin, crocetin and digoxin, respectively. The flow rate of nebulizing gas was optimized at 2 L/min, and the heating gas was set at 15 L/min.

### 2.6. Validation of the LC–MS/MS method

#### 2.6.1. Specificity

Blank plasma samples (from six different batches) were prepared, extracted, and analyzed for determining any endogenous molecules co-eluted. The effect of endogenous interference on the assay of crocin, crocetin and IS was assessed.

#### 2.6.2. Linearity and sensitivity

Linearity of both crocin and crocetin (at nine concentrations of 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL) were assessed by linear regressing after 1/x weighting of the peak area ratio of analyte/IS versus the concentrations of the two analytes. Lower limit of quantification (LLOQ) was examined based on the signal-to-noise ratio at 10. The precision was calculated and expressed by relative standard deviation(RSD) and the accuracy was calculated by relative error (RE), which were not more than 20%.

#### 2.6.3. Precision and accuracy

To evaluate the precision and accuracy, six replicates of spiked plasma samples with known concentrations of crocin and crocetin at three concentrations (5, 100, 1000 ng/mL) were analyzed. The intra-day precision and accuracy were examined in one day as above at each of the three concentration levels, while the inter-day precision and accuracy of the assays were determined on three consecutive days.

#### 2.6.4. Recovery and matrix effect

The recoveries of crocin and crocetin from blank plasma samples of rats were assessed by comparing the peak area of the two analytes spiked and extracted from blank plasma with these of extracted from blank plasma samples and then spiked with the two analytes at three concentrations (5, 100, 1000 ng/mL, n = 6). IS (2 μg/mL) was examined in same way.

Blank rat plasma and deionized water were prepared, extracted and then spiked with the analytes at 5, 100, 1000 ng/mL, respectively. The resulted peak areas ratios were calculated to evaluate the matrix effect. IS was also determined at the concentration of 2 μg/mL.

#### 2.6.5. Stability

The stability of crocin and crocetin was evaluated after in autosampler vials (24 h), short-term storage (6 h) at room temperature, 3 times freeze-thaw cycles and 2 weeks storage at −80 °C at 5, 100, 1000 ng/mL, respectively, in rat plasma.

#### 2.6.6. Dilution integrity

Crocin and crocetin samples with the concentration of 0.05, 1, 8 μg/mL were diluted for 10 times with blank plasma. The diluted