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Micelle-mediated extraction and cloud-point preconcentration of osthole and imperatorin from *Cnidium monnieri* with analysis by high performance liquid chromatography

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ABSTRACT

A new method based on micelle-mediated extraction and cloud-point preconcentration was developed for the separation and determination of hydrophobic compounds osthole and imperatorin from *Cnidium monnieri* by high performance liquid chromatography with photodiode array detection. The non-ionic surfactant $C_{13}E_8$ (Genapol X-080) was chosen as the extract solvent. Various experimental conditions were investigated to evaluate and optimize the extraction and preconcentration process. The chromatographic separation was accomplished on a Zorbax SB-C_{18} analytical column (150 mm × 4.6 mm i.d., 5 μm particle diameter) maintained at 30 °C and detected by UV absorption at 320 nm. The gradient elution was achieved with a mobile phase composed of 0.1% phosphoric acid and acetonitrile at a flow rate of 1.0 mL min^{-1}. Under the optimum conditions, the calibration curve for both analytes was linear in the range of 0.52–33.5 μg mL^{-1} with the correlation coefficients greater than 0.9996. The intra-day and inter-day precision (RSD) is below 5.3% and the limits of detection (LOD) for the analytes are 93 and 124 ng mL^{-1} (S/N = 3). The proposed technique is a low cost, simple and sensitive method with high clean-up effect. Finally, the method was successfully applied to separate and determine osthole and imperatorin from *C. monnieri*, respectively.

1. Introduction

The fruit of *Cnidium monnieri* (Chinese name: She Chuang Zi) [1,2] has been widely used in traditional Chinese medicine over hundreds of years as a remedy for arthritis, lumbago, edema, headache, common cold, thrombosis, stasis of blood [3], skin disease and gynecopathy [4]. Osthole and imperatorin are the main effective constituents of *C. monnieri*, which possesses a variety of pharmacological and biochemical properties of the central nervous, cardiovascular, endocrine and immune systems [3]. These two compounds have coumarin as their parent structure (as shown in Fig. 1).

As the clinical use of osthole and imperatorin became common, methods for their quantification have attracted the attention of many investigators. The most widely used methods for analyzing osthole and imperatorin are chromatographic techniques such as gas chromatography (GC) [5,6] or high performance liquid chromatography (HPLC) [7,8], but their sensitivity and selectivity limit their direct use for determination of osthole and imperatorin at a very low concentration level in biological fluids with a complex matrix. Therefore, a sample pretreatment step prior to chromatographic analysis is usually necessary, such as liquid–liquid extraction (LLE) [9,10], solid-phase extraction (SPE) [11–13] and solid-phase microextraction (SPME) [14]. Unfortunately, all of these methods require a large sample volume and are time-consuming. In particular, the traditional liquid–liquid extraction method is also dangerous to analysts because of the large amounts of toxic and volatile organic solvents required. So, a simple and environmentally friendly method should be established.

The micelle-mediated extraction and cloud-point preconcentration (CPE) method offers a convenient alternative to the conventional extraction systems [15,16]. The micelle-mediated extraction process can be divided into two parts: the first step is to solubilize and purify analytes into the aqueous surfactant solution; the second step is to preconcentrate analytes based on phase separation by the cloud-point methodology. When the temperature rises above the cloud-point temperature, the solution separates into two phases: the small volume of surfactant-rich phase and the

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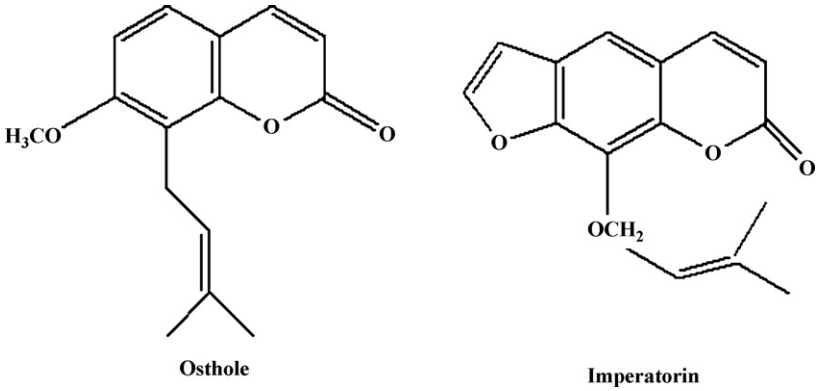

Fig. 1. Chemical structures of osthole and imperatorin.

large volume of aqueous phase. The small volume of the surfactant-rich phase allows us to preconcentrate the analytes [17–22]. This methodology offers the advantages of safety, low cost, ability to concentrate solutes, easy disposal of surfactant, and low toxicity compared with classical organic solvents, etc. [23]. Up until now, the majority of cloud-point extraction (CPE) applications have been reported to deal with analytes present in aqueous samples, including estrogens, vitamin A, vitamin E, kinds of proteins, as well as metal ions [24–29]. All these indicate that CPE has great analytical potential as an effective enrichment method. But CPE has rarely been reported to be used for extraction and preconcentration of chemical constituents from solid materials, especially plants or herbal materials; no reports have been published about how to extract osthole and imperatorin from *C. monnieri* by CPE.

In this paper, the application potential of the micelle-mediated extraction and cloud-point preconcentration method has been further evaluated by employing non-ionic surfactant Genapol X-080 for the extraction and preconcentration of osthole and imperatorin from *C. monnieri*. Prior to determining osthole and imperatorin by means of HPLC, the extraction and preconcentration method established in this work includes two steps: the first step is to extract osthole and imperatorin from solid herbal materials into aqueous surfactant solution; the second step is to preconcentrate them by phase separation based on the cloud-point phenomenon of the surfactant. To optimize the micelle-mediated extraction step, concentration of the surfactant solution, liquid/solid ratio and ultrasonic-assisted extraction time were all investigated. For the preconcentration of osthole and imperatorin by CPE, the effects of the equilibration time and temperature as well as the amount of electrolyte on the recovery of the analytes were all studied. After optimization and validation, the method has been applied to the separation and determination of osthole and imperatorin from *C. monnieri*.

2. Experimental

2.1. Plant materials

Dried fruit of *C. monnieri* was purchased from a local pharmaceutical store (Xi'an, China). The dried plant materials were pulverized and sieved to produce samples with particle sizes up to 65 mesh (250 μm). The dried powder was stored in a moisture-controlled cabinet.

2.2. Chemicals and reagents

Authentic standards of osthole and imperatorin were obtained from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China). Non-ionic surfactant $C_{13}E_8$ (Genapol X-080) was obtained from Fluka (USA) and used without further purification. Various concentrations (v/v) of aqueous surfactant solutions were prepared by weighing appropriate amounts of the surfactant and by directly dissolving the surfactant in distilled water. Sodium chloride (Beijing Chemical Factory, AR China) was prepared before each experiment. Acetonitrile and phosphoric acid were of HPLC grade. All other reagents used in this work were of analytical grade. Distilled water (Millipore, Bedford, MA, USA) was used throughout the study.

2.3. Instrumentation

The analyses were completed using an Agilent 1100 series HPLC system, including a quaternary pump, a photodiode array detector (G1315B), a column oven and a Rheodyne 7225i injector. The analytical column was an Agilent Zorbax SB-C_{18} (150 mm × 4.6 mm i.d., 5 μm particle diameter) column connected to an Agilent Zorbax Extend-C_{18} guard column (12.5 mm × 2.1 mm i.d., 5 μm particle diameter). The column temperature was controlled at 30 °C. A personal computer equipped with an Agilent Chemstation program for LC systems was used to acquire and process chromatographic data. Peak area was evaluated as the analytical measurement.

A versatile plant pulverizer (Tianjin, China) was used to make the plant materials into powder. Sieves (Zhejiang, China) were used to sieve the *C. monnieri* powder. An SB 3200 ultrasonic generator (50 kHz, 300 W) from the Shanghai Branson Ultrasonics Co. Ltd. (Shanghai, China) was used to extract osthole and imperatorin from the samples. A vortex mixer (WH-861 tai cang) was used to blend the solution adequately. A thermostatic bath (HH-2, Guohua Medical Instrument Company, PR China), set at the desired temperature, was used to implement cloud-point extraction. High-speed centrifugation (Model 800, Shanghai, China) was employed to accelerate the phase separation process.

2.4. Procedures

2.4.1. Micelle-mediated extraction procedure

Powders of *C. monnieri* (0.1 g) were accurately weighed and placed in a 25 mL centrifuge tube, and 20 mL Genapol X-080 solution (0.5–20%) (v/v) was added. The tube was capped and blended adequately, then placed in the ultrasonic cleaning bath for ultrasonic extraction (300 W) for 30 min at room temperature (22 °C). After ultrasonic-assisted extraction, the *C. monnieri* extracts were centrifuged at 3500 rpm (1120 × *g*) for 10 min, the supernatant was filtered through a 0.45 μm membrane and 20 μL of the solution was injected into the HPLC system for analysis.