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Original Article

Optimization of ultrasonic extraction by response surface methodology combined with ultrafast liquid chromatography–ultraviolet method for determination of four iridoids in *Gentiana rigescens*

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ABSTRACT

Gentiana rigescens is a rich source of iridoids and is commonly used as a folk medicine for treatment of hepatitis and cholecystitis for over 1000 years. A rapid ultrafast liquid chromatography–ultraviolet method was developed for simultaneous determination of four major iridoid glycosides in *G. rigescens*. Response surface methodology based on the Box–Behnken design was applied to optimize the extraction conditions of iridoid glycosides. Using the Shim-Pack XR-ODS III, four iridoid glycosides were efficiently separated with an acetonitrile:0.1% formic acid aqueous solution gradient at a flow rate of 0.25 mL/min for 8 minutes. All the regression equations revealed a good linear relationship ($R^2 > 0.9995$). The intraday and interday variations were <1.95%. The recoveries ranged from 99.7% to 103.2%. The optimal extraction conditions were as follows: methanol concentration, 82%; the ratio of liquid to solid material, 68:1 (mL/g); and extraction time, 32 minutes. The yield of the four iridoid glycosides under the optimal process was found to be 63.08 mg/g, which was consistent with the predicted yield. In addition, the total content of 50 cultivated samples from Lincang, Yunnan, China, was within the range of 33.6–113.26 mg/g, which provides a more reasonable foundation for utilization of *G. rigescens*.

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1. Introduction

Herbal medicines have been widely used as folk medicines since ancient times to treat various diseases and improve health conditions. The quality of these medicines has captured worldwide attention. However, many factors such as sample preparation, target compounds, and analytical methods have a significant impact on the quality evaluation of herbal medicines [1,2].

Gentiana rigescens (Family: Gentianaceae) is a perennial species, which is mainly distributed in the Yunnan, Sichuan, Guizhou, Hunan, and Guangxi provinces in China. It is usually found in grassland slopes growing at elevations of 1100–3000 m [3]. The roots and rhizomes of *G. rigescens*, *Gentiana scabra*, and *Gentiana triflora* are recorded as the source materials of the important traditional Chinese medicine "Long Dan," which is commonly used as a hepatoprotective agent and as an anti-inflammatory agent in Chinese pharmacopoeia [4]. Modern phytochemical and pharmacological studies reported that *G. rigescens* is a rich source of iridoids. Among them, gentiopicroside, loganic acid, swertiamarin, and sweroside are the major chemical constituents and are commonly considered as the main indexes for quality evaluation of "Long Dan" [5–8]. According to a previous study, the total content of the four iridoid glycosides in *G. rigescens* was >4.5% [6].

Because of habitat loss and overharvesting, the numbers of wild grown *G. rigescens* are significantly reduced [9–11]. As *G. rigescens* is a major raw material of "Long Dan," the species is widely cultivated in Yunnan, China. The cultivated *G. rigescens* species is used as a supplement to the wild grown species in the preparation of "Long Dan" [5]. To accurately evaluate the constituents of *G. rigescens*, it is necessary to optimize the extraction of the four iridoid glycosides.

Response surface methodology (RSM) is a well-established tool for the optimization of analytical methods owing to its advantages over classical one-variable-at-a-time optimization [12]. At present, sample preparation by RSM is widely applied for analysis of foods and herbal medicines [13–19]. Wang et al [13] used RSM to optimize extraction conditions of polysaccharides from *G. scabra* to evaluate their antioxidant and antitumor activities *in vivo*. Liang et al [14] developed a high-speed counter-current chromatography method combined with RSM, which could effectively separate and extract six bioactive compounds from *Gentiana crassicaulis*.

In the present study, a rapid and reliable ultrafast liquid chromatography–ultraviolet (UFLC–UV) method for quantification of the four iridoid glycosides (i.e., loganic acid, swertiamarin, gentiopicroside, and sweroside) was developed and validated. Then, three main independent variables including methanol concentration, ratio of liquid to material, and extraction time were studied. Three-factor and three-level RSM based on the results of single-factor experiments were used to optimize the process variables of the four iridoid glycosides extracted from *G. rigescens*. Moreover, 50 samples were tested using the developed method for quality evaluation of cultivated *G. rigescens* collected from a major production area (Lincang, Yunnan, China).

2. Materials and methods

2.1. Materials and reagents

The roots and rhizomes of *G. rigescens* (50 samples) were collected from Lincang, Yunnan province of China in November 2012 and authenticated by Professor Hang Jin (Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming, China).

The high-performance liquid chromatography (HPLC)-grade solvents (acetonitrile and formic acid) were purchased from Tedia (Fairfield, USA) and Dikmapure (Lake Forest, USA), respectively. The methanol (Tianjin Feng Chuan Fine Chemical Research Institute, Tianjin, China) for extraction is of analytical grade. The pure water used in experiments was purified using a Milli-Q system (Millipore, Billerica, USA). The standards (1, loganic acid; 2, swertiamarin; 3, gentiopicroside; and 4, sweroside shown in Fig. 1) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All markers were determined to be of >98% purity by UFLC–tandem mass spectrometry (UFLC–MS/MS). The stock solutions of each marker were prepared in methanol by weighing them accurately and separately, and were finally stored at 4°C.

2.2. Apparatus

The HPLC system (Shimadzu Technologies, Kyoto, Japan) was equipped with an SPD-M10A VP photodiode array detector. Data acquisition was performed in the range of 200–400 nm.

The UFLC–MS/MS (LCMS-8030; Shimadzu, Kyoto, Japan) was equipped with an autosampler, binary gradient pumps, UV detector, and triple quadrupole mass analyzer with an

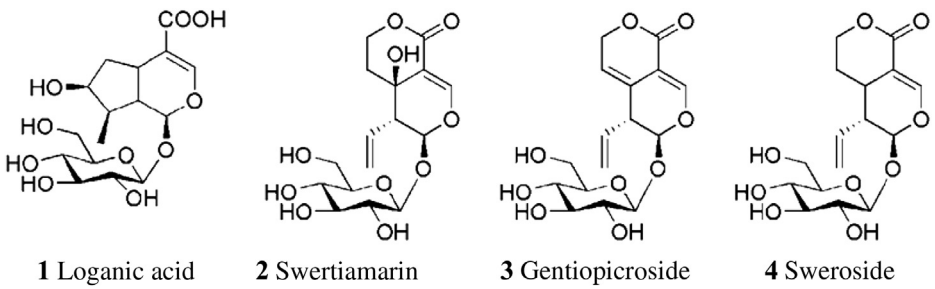

Fig. 1 – Structures of the four iridoid glycosides: 1, loganic acid; 2, swertiamarin; 3, gentiopicroside; 4, sweroside.