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坚龙胆环烯醚萜苷的 HPLC 分析

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摘 要:应用 HPLC 分析方法,建立了坚龙胆中 5 个主要环烯醚萜苷的含量测定方法。在 ZORBAX SB-C_{18} 色谱柱,乙腈-0.2%磷酸水溶液为流动相,梯度洗脱,流速 1.0 mL/min,柱温为 40 ℃,检测波长 254 nm 的色谱条件下,化合物得到良好的分离,并具线性相关。本方法操作简便,重现性好,灵敏度高。对不同产地的样品进行比较分析的结果表明,产地对坚龙胆中环烯醚萜苷的组成和含量有显著的影响。

关键词:坚龙胆;环烯醚萜苷;HPLC 分析

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HPLC Analysis of the Iridoidal Glycosides in *Gentiana rigescens*

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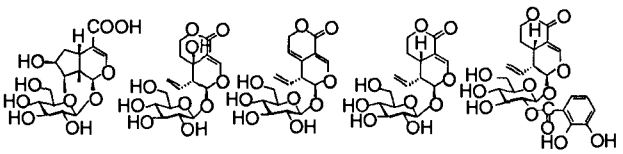
Abstract: A rapid, sensitive and selective HPLC method was established to determine the content of iridoidal glycosides in the root of *G. rigescens*. The MeOH extract of the plant material was analyzed on a reversed-phase column ZORBAX SB-C_{18} (150 × 4.6 mm, 1.5 m) under column temperature 40 ℃, with a mobile phase consisted of acetonitrile and 0.2% acetic acid aqueous solution (8:92, v/v). The optimal flowing rate of mobile phase was 1.0 mL/min and a selective detection was at 254 nm. The simultaneous separation was realized for loganic acid (**1**), swertiamarin (**2**), gentiopicroside (**3**) sweroside (**4**), and 2′-(o,m- dihydoxybenzyl)-sweroside (**5**) in the root of *G. rigescens*. The variation of iridoidal glycosides of *G. rigescens* collected from different areas was discussed.

Key words: *Gentiana rigescens*; iridoidal glycosides; HPLC analysis

Introduction

"Long-Dan" is a well-known herb in traditional Chinese medicine (TCM) commonly used for treatment of inflammation, hepatitis, rheumatism, cholecystitis and tuberculosis. In the Chinese pharmacopoeia, the roots of *Gentiana scabra*, *G. manshurica*, *G. triflora and G. rigescens* are used as the raw materials of "Long-Dan"[1]. Among them, *G. rigescens* mainly grows in the southwest China, particularly in Yunnan Province that called as "Jian-Long-Dan".

It is well know that *G. rigescens* contains rich iridoidal glycosides, and gentiopicroside (**3**), a secoiridoidal glycoside, is a major constituent in this herb [2,3]. As one of our systematic phytochemical investigation on the medicinal plants of genus *Gentiana*, thirty-five compounds were isolated from the roots of *G. rigescens*. Among them, iridoidal glycosides are the main constituents [4]. The present paper reported on analyzing contents of five major iridoidal glycosides, loganic acid (**1**), swertiamarin (**2**), gentiopicroside (**3**) sweroside (**4**) and 2′-(o,m-dihydoxybenzoyl)-sweroside (**5**) of this plant by means of HPLC analysis. The content of iridoidal glycosides in different geographic distributions of *G. rigescens* was determined too.



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Materials and Methods

Chemicals

Loganic acid (**1**), swertiamarin (**2**), gentiopicroside (**3**) sweroside (**4**), and 2′-(o,m-dihydoxybenzoyl)-

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sweroside (**5**) were isolated from *G. rigescens*. The purity and structure identity were carried out by our previous work[4]. HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Analytical grade acetic acid was obtained from Shanghai Chem. Co. (Shanghai, China).

Samples and preparation

The roots of *G. rigescens* were collected from different areas of Yunnan Province: A (Kunming); B (Heqing alt. 2000 m); C (Chuxiong); D (Heqing, alt. 3000 m); E (Dali), F (Wenshan); G (Yongsheng) and H (Linchang). The powdered drying plant material (0.5 g) was extracted with MeOH (10 mL) under ultrasonic condition for 30 min. The extracted solutions were filtered through a 0.45 μm nylon filter membrane. After made up a weight lost with methanol, an aliquot of each filtrate (10 L) was injected for HPLC analysis.

HPLC analysis

The HPLC analysis was performed on a Shimadzu LC-6A pump (Kyoto, Japan) equipped with a variable-wavelength Model LC-65T UV detector using a ZORBAX SB-C_{18} analytical column (150 × 4.6 mm, 1.5 μm) [mobile phase: acetonitrile and 0.2% acetic acid aqueous solution (8:92, v/v); flow rate, 1.0 mL/min; column temperature, 40 ℃]. Data were collected at 254 nm and peaks were identified by co-chromatography with authentic samples, according to the UV spectral data and retention times.

Results and Discussion

Solvent extraction

At first, the condition of extraction was examined. When the samples were extracted with MeOH under ultrasonic condition for 30 min, maximal extracted yield of iridoidal glycosides from samples were achieved. More solvents were chosen for extraction and methanol was found to be the best solvent for the extraction of iridoidal glycosides.

Optimization of separation

The effect of varying the composition of solvent was investigated. The following mobile systems were tried: aqua and acetonitrile or methanol. The pH of the solvent was modified by acetic acid. It was found that pH of the solvent has a significant influence on the retention of all peaks. After taking the tried separation systems into consideration, the best separation of five iridoidal glycosides was achieved using acetonitrile and 0.2% acetic acid aqueous solution (8:92, v/v) as mobile phase.

Optimization of detection

Determination of iridoidal glycoside 1 together with its related glycosides seemed to be difficult due to instability, interference and complexity of constituents in the plant materials. We focused on the influence of mobile phase on detection response, column temperature and flow rates. Through the selective tests, better result of the analysis of iridoidal glycosides was achieved by the reverse-phase column ZORBAX SB-C_{18} (150 × 4.6 mm, 1.5 m). Mobile phase consisted of acetonitrile and 0.2% acetic acid aqueous solution (8:92, v/v). Flow rate of mobile was maintained at 1.0 mL/min under column temperature 40 ℃, and selective detection was at 254 nm.

Calibration curves and its validation

Calibration curves were plotted by correlating the peak area ration versus responding concentration ratios. The linear range was 8, 10, 12, 14, 16, 18, 20, 22, 24 g/mL for compound 1; 1, 2, 4, 6, 8, 10, 12, 14 g/mL for compound 2; 0.5, 1, 2, 4, 6, 8, 10, 12, 14 g/mL for compound 3; 1, 2, 4, 6, 8, 10, 12, 14 g/mL for compound 4; 0.5, 1, 2, 4, 6, 8, 10, 12, 14 g/mL for compound 5, respectively. Standard solutions were injected three times for each concentration level. Least-squares linear regression analysis of the data demonstrated good linearity over the examined range with R^2 value exceeding 0.999. Calibration curves were plotted by correlating the peak area ration versus and shown in Fig 1. The precision (expressed in relative standard deviation, n = 5) of standard solution of each compounds (1 ~ 5) were determined as 1.35%, 3.21%, 1.33%, 2.22% and 3.33%, respectively. Moreover, recoveries and repeatability of five iridoidal glycosides were determined as 101%, 90%, 92%, 102% and 90%; and 0.74%, 0.80%, 0.25%, 93% and 0.86%, respectively.

Based on above results, typical chromatograms of a methanol extract of the roots of *G. rigescens* under proposed chromatographic conditions are shown in Fig. 2.

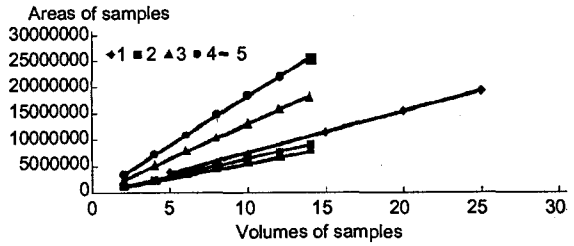

Fig. 1 The calibration curves of iridoidal glycosides

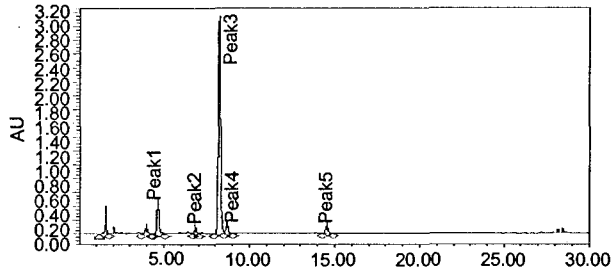

Fig. 2 Chromatographic profile of the methanolic extract of the root of *G. rigescens*

Peak 1: Loganic acid; Peak 2: Swertiamarin; Peak 3: Gentiopicroside; Peak 4: Sweroside; Peak 5: 2′-(o, m-dihydroxybenzoyl)-sweroside.

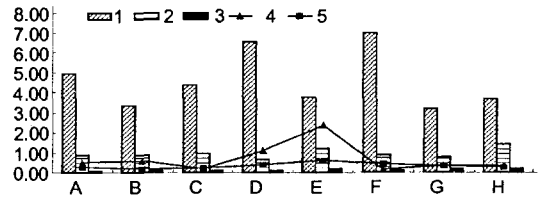

Fig. 3 Contents (w/w, %) of iridoidal glycosides 1 ~ 5 in the root of *G. rigescens* a)

a) The root samples (A-H) were collected from different places of Yunnan province, China [A. Kunming; B. Heqing (alt. 2000 m); C. Chuxiong; D. Heqing (alt. 3000 m); E. Dali; F. Wenshan; G. Yongsheng; H. Linchang]

Conclusion

Under the suggested conditions, a good separation was achieved. The proposed HPLC method was suitable for determination of the composition and quantitative analysis of main iridoidal glycosides in the crude material and preparation of *G. rigescens*. Recently, Jiang et al. have been reported the HPLC determination of Radix gentianae and quantitative analysis of gentiopicroside (**3**) and sweroside (**4**) by using gradient mobile phase[5]. However, our proposed method was considered as appropriate for quantitative analysis of the main component of this herb. It took shorter time (20 min) with isocratic elution and the five major iridoidal glycosides in *G. rigescens*, loganic acid (**1**), swertiamarin (**2**), gentiopicroside (**3**) sweroside (**4**), and 2′-(o, m-dihydoxybenzoyl)-sweroside (**5**), could be studied simultaneously for the qualitative and quantitative analysis, which exhibited excellent separations as showed in Fig. 2. The proposed analytical method is more rapid, sensitive, accurate, reproducible, and convenient. It can be readily utilized as a suitable quality control method for *G. rigescens*.

With this proposed HPLC analytical method, several samples collected from different places of Yunnan, China were determined and the result was shown in Fig 3.

It is noticed that the content of compound 1, the main constituent in the root, was ranged from 3.18% to 7.00%.

And the other four glycosides were also showed distinct content changes from different origins. The analytic results suggested that the accumulation and composition of iridoidal glycosides in *G. rigescens* may correlation with geographic distribution. A further investigation for the variation of iridoidal glycosides in the population of *G. rigescens* is now in progress.

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