·制剂与质量·

# Determination of marker compounds in plants of Rhodiola L. from different habitats by RP-HPLC

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**Abstract: Object** To develop the reliable RP-HPLC methods for the determination of salidroside, tyrosol, rosavin, rosin, and rosarin in the plants of *Rhodiola* L. and to evaluate their species from different habitats. **Methods** Method Ⅰ: methanol-water (0.5 mmol/L SDS in 1% acetic acid aqueous solution) system for the analysis of salidroside; method Ⅱ: acetonitrile-water system for rosavin; method Ⅲ: aqueous acetonitrile-phosphoric gradient system for salidroside, tyrosol, rosavin, rosin, and rosarin. **Results** The contents of salidroside in different species range from 0.021% to 1.420%, and those of rosavin in all species are very limited or undetected except in *Rhodiola rosea* L. and *R. sachalinensis*. The contents of the five marker ingredients are significantly species- and habitat-dependent. **Conclusion** Three RP-HPLC methods are established for quantitative analysis of the above five marker ingredients in the meantime, respectively. Evaluation of the quality of varied species of *Rhodiola* L. shows that *R. rosea* growing in Xinjiang Uygur Autonomous Region and *R. sachalinensis* growing in Jilin province are the two better species contained with abundant above-mentioned ingredients in China.

**Key words:** *Rhodiola* L.; salidroside; tyrosol; rosavin; rosin; rosarin; RP-HPLC

## RP-HPLC 法测定不同品种和产地红景天中指标成分的含量

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**摘 要: 目的** 建立快速、有效的反相高效液相(RP-HPLC)检测方法, 测定不同品种和产地红景天中红景天苷、红景天芬、酪醇、红景天素、红景天任的含量, 以评价红景天的质量。**方法** 方法Ⅰ: 甲醇-水(0.5 mmol/L SDS 的 1% 醋酸水溶液)检测红景天苷; 方法Ⅱ: 乙腈-水检测红景天芬; 方法Ⅲ: 乙腈-磷酸水溶液梯度洗脱同时检测 5 个有效成分。**结果** 红景天苷的含量为 0.021%～1.420%; 红景天芬除在蔷薇红景天和高山红景天中含量较高外, 在其他品种中均非常低或低于检测限; 5 个有效成分的含量因品种或产地的不同而有很大的变化。**结论** 建立的 3 种 RP-HPLC 方法可同时测定红景天苷、红景天芬等 5 个有效成分。综合考虑不同品种中 5 个有效成分的含量, 以产自新疆的蔷薇红景天和吉林的高山红景天为国内较好的品种。

**关键词:** 红景天属; 红景天苷; 酪醇; 红景天芬; 红景天素; 红景天任; 反相高效液相色谱

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## 1 Introduction

The *Rhodiola* L. plant contains about 90 species and their various species naturally display a circumpolar distribution in mountainous regions with higher latitudes and elevations of the Northern Hemisphere[1]. Traditional folk medicine, plants of *Rhodiola* L., especially *R. rosea*, used to increase physical endurance, work productivity, longevity, resistance to high latitude sickness, and to treat fatigue, depression, nervous system disorders, *etc*[2～5]. Since 1960, a variety of *Rhodiola* products have been offered and consumed as adaptogens with various health-promoting effects. With dramatically increase of demand for *Rhodiola*-

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based phytomedicines in the late 1980s, and because of their significant species-dependent variation in phytochemistry and pharmacology, the standardization of *Rhodiola* products or extracts is of great importance. Scientific investigation revealed that two groups of chemical constituents were responsible for the unique pharmacological properties of *Rhodiola* products: one is phenylpropanoids including rosavin, rosin, and rosarin[6,7], and the other is phenylethanol derivatives including salidroside and tyrosol. Therefore, the *Rhodiola* extracts or preparations are usually standardized for both rosavin and salidroside to guarantee their quality and effectiveness.

China has abundant resources of plants of *Rhodiola* L., and nearly 80 species of them are distributed in China. The analysis of *Rhodiola* products is reported only for salidroside, and the standardization by determination of rosavin has not been reported in China. Moreover, none of the reported HPLC methods, the detection of salidroside, and tyrosol in *Rhodiola* products[8,9] was satisfactory. Thus, in our ongoing effort to solve the above-mentioned problems, three HPLC methods were developed for the quantitative analysis of the five marker compounds, such as salidroside, rosavin, tyrosol, rosin, and rosarin in one run, respectively.

## 2 Experiment

### 2.1 Preparation of stock solutions of reference

Salidroside and rosavin (ChromaDex), 2 mg for each were accurately weighed, and dissolved in two 10 mL volumetric flasks with methanol as stock solution for method Ⅰ and Ⅱ, respectively.

Salidroside, tyrosol, rosavin, rosin, and rosarin, 1 mg for each, were accurately weighed, mixed together, and dissolved in a volumetric flask with methanol as stock solution for method Ⅲ.

### 2.2 Calibration curve

The stock solutions were diluted 2, 4, 8, 12, 16 times in methanol, respectively. Using Anastar chromatography data system, all these standard were injected to generate a five-point calibration curve for the three methods respectively. Standard curve, as shown in Table 1, was linear with $R^2$ not less than 0.998 6.

### 2.3 Sample preparation

The plant materials of *Rhodiola* L. were collected from wild location in China. All the samples were identified by Yu Hong-jian, a senior engineer from Tianjin TCM Factory. Three extracting methods including ultrasonic extraction, Soxhlet apparatus extraction, and room temperature extraction were compared. Results showed that ultrasonic extraction was a simple, fast, and efficient method. The roots of each species (about 200 g) were finely powdered, 0.5 g of each samples was accurately weighed, ultrasonically extracted with 30 mL of 60% aqueous ethanol for 20 min three times, extraction solutions were centrifuged at 5 000 r/min for 15 min, supernatants were combined and adjusted to a 100 mL volumetric flask with 60% aqueous ethanol. All sample solutions were filtered through a 0.45 μm Acrodisc syringe filter before being subjected to HPLC analysis. Each sample solution (10 μL) was injected for HPLC analysis.

**Table 1 Calibration data for each reference compound in method Ⅰ—Ⅲ**

| Methods | Compounds | $R^2$ | Regression equation |
|---|---|---|---|
| Ⅰ | salidroside | 0.999 1 | $Y = 1.56 \times 10^4 X$ |
| Ⅱ | rosavin | 0.999 5 | $Y = 2.58 \times 10^4 X$ |
| Ⅲ | salidroside | 0.999 2 | $Y = 1.44 \times 10^4 X$ |
| | tyrosol | 0.999 1 | $Y = 3.67 \times 10^4 X$ |
| | rosavin | 0.999 3 | $Y = 2.31 \times 10^4 X$ |
| | rosin | 0.998 6 | $Y = 2.42 \times 10^4 X$ |
| | rosarin | 0.998 5 | $Y = 2.41 \times 10^4 X$ |

$Y$ reflects peak area, $X$ reflects amount of compound in μg/mL

### 2.4 Stability test

Each sample solution (10 mL) was injected into HPLC in each method after 1, 3, 5, 7, 12 h, respectively. Results showed that all the relative standard deviation (RSD) were less than 3.00% and indicated the sample solutions were stable within 12 hours.

### 2.5 Recovery test

The recovery was determined by adding known amounts of each reference compound to the plant sample prior to the extraction, and all the samples were dealt with the same method as described in Section 2.3. The determined concentration of each ingredient was divided by the added concentration, and the recoveries were calculated in percentages. The added concentrations were based on the mean concentration of these ingredients in three unspiked samples. RSD was less than 3.01%, and average recovery ratio was not less than 95.0%.

## 3 Analytical methods

HPLC analysis was performed on a Shimadzu

LC—10A Tvp system equipped with Shimadzu SPD—10A vp detector. For the methods Ⅰ and Ⅱ, a Phenomenex $C^{18}$ column (250 mm × 4.6 mm, 5 μm) was used, and for method Ⅲ a Shimadzu VP—ODS column (150 mm × 4.6 mm, 5 μm) was used.

Mobile phase in method Ⅰ: Methanol-water solution (0.5 mmol/L SDS 1% acetic acid water solution) (25∶75); flow rate: 0.8 mL/min; detection wavelength: 225 nm.

Mobile phase in method Ⅱ: Acetonitrile-water (25∶75); flow rate: 1.0 mL/min; detection wavelength: 254 nm.

Mobile phase in method Ⅲ: Solution A: 0.2% aqueous phosphoric acid, solution B: Acetonitrile, the solvent gradient was A, initial 96% 20 min 70%; 25 min 96%; 30 min 96% (run time 30 min); detection wavelength: 1—12 min 225 nm, 12—30 min 254 nm; flow rate: 0.8 mL/min. Each sample (10 μL) was injected. All separation was at room temperature. Peaks were assigned by spiking the samples with reference compounds. The results can be seen in Table 2 and Fig. 1—3.

**Table 2 Analysis of different species of** Rhodiola **raw materials in China and extracts from Tianjin Jianfeng Natural Product R&D Co., Ltd (values in g/100 g)**

| Species | Habitats (Provinces) | Time | Salidroside | Tyrosol | Rosavin | Rosin | Rosarin |
|---|---|---|---|---|---|---|---|
| *Rhodiola rosea* | Xinjiang | June 2002 | 1.232 | 0.132 2 | 1.621 | 0.311 | 0.214 |
| *R. sachalinensis* | Jilin | July 2001 | 0.351 | 0.084 5 | 0.212 | 0.101 | 0.084 |
| *R. sachalinensis* | Yunnan | June 2001 | 0.241 | 0.001 4 | 0.014 | - | - |
| *R. crenulate* | Yunnan | July 2001 | 1.420 | 0.231 1 | 0.032 | 0.008 | - |
| *R. crenulate* | Sichuan | Sep. 2001 | 0.411 | 0.054 1 | - | - | - |
| *R. crenulate* | Qinghai | Sep. 2001 | 1.024 | 0.084 2 | - | - | - |
| *R. kirilowii* | Qinghai | Sep. 2001 | 0.667 | 0.023 4 | - | - | - |
| *R. fastigiata* | Yunnan | July 2001 | 0.021 | - | - | - | - |
| *R. alterna* | Tibet | Sep. 2001 | 0.321 | 0.021 4 | 0.012 | - | - |
| *R. rosea* extract | Xinjiang | 011223 | 3.584 | 0.524 1 | 6.745 | 1.412 | 1.110 |
| *R. crcnulata* extract | Yunnan | 020102 | 4.521 | 0.642 1 | 0.122 | 0.021 | - |
| *R. coccinea* extract | Qinghai | 020203 | 4.661 | 0.682 3 | - | - | |

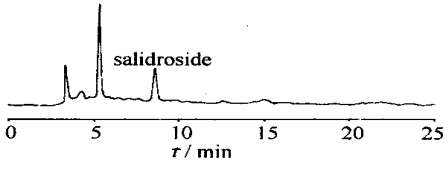



**Fig. 1 Chromatogram of salidroside (method Ⅰ)**

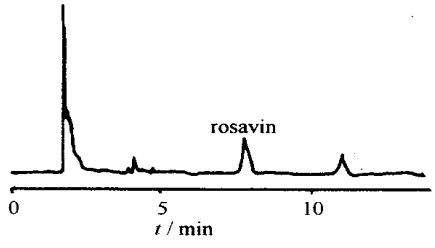
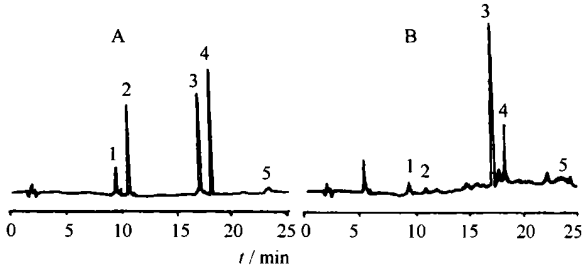



**Fig. 2 Chromatogram of rosavin (method Ⅱ)**




1-salidroside 2-tyrosol 3-rosavin 4-rosavin 5-rosin

**Fig. 3 Chromatogram of five reference substances (A), R. rosea raw material (B), and separated by method Ⅲ**

## 4 Results and discussion

The developed method Ⅲ permitted the first simultaneous detection of both "salidrosides" (salidroside and tyrosol) and "rosavins" (rosavin, rosin, and rosarin). Yet the RP-HPLC condition was somewhat complicated because of the gradient mobile phase, changing detection wavelength and relatively longer running time. Thus, method Ⅰ for salidroside and method Ⅱ for rosavin have also been developed in order to meet the demand for the detection of different types of marker ingredients. These three methods are proved to be rapid, sensitive, and reliable HPLC methods for the evaluation of *Rhodiola* species and their related extract or products.

The results of the determination of the marker ingredients of different *Rhodiola* species and the market extracts available in China showed that their contents are significant species-dependent and also influenced by the wild location even for the same species. In conclusion, *R. rosea* from the

Xinjiang Uygur Autonomous Region and *R. sachalinensis* from Jilin Province are two species with higher contents of the marker ingradients.

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# 超声循环提取灵芝中三萜类化合物的研究

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**摘 要:** **目的** 研究超声循环技术在灵芝中三萜类化合物提取中的应用。**方法** 在常规提取方法的基础上, 增加超声循环的处理步骤。**结果** 通过试验对比, 超声循环提取所需各种溶剂用量减少, 提取时间缩短, 目的产物提取率提高了 40%。与常规方法提取得到的目的产物之间存在着良好的相关性。**结论** 超声循环技术用于灵芝中三萜类化合物的提取具有良好的应用前景。

**关键词:** 灵芝; 三萜类化合物; 超声循环技术; 高效液相色谱

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## Study on ultrasonic circulation technique to extraction of triterpenoids from Ganoderma lucidum

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**Abstract:** **Object** To study the application of ultrasonic circulation technique for the extraction of triterpenoids from *Ganoderma lucidum* (Leyss. ex Fr.) Karst. **Methods** On the basis of conventional method, the processing steps of ultrasonic circulation treatment were added. **Results** The comparative experiments showed that less amounts of various solvents and shorter extraction time were needed for ultrasonic circulation extraction, with the product ratio of about 40 percent higher than that of conventional method. Furthermore, a good consistency of the target product analyzed by HPLC was found between two different extraction methods. **Conclusion** The ultrasonic circulation technique has a potential application to the extraction of triterpenoids from *G. lucidum*.

**Key words:** *Ganoderma lucidum* (Leyss. ex Fr.) Karst.; triterpenoids; ultrasonic circulation technique; HPLC

灵芝在我国已有两千多年的药用历史。近年来, 从灵芝的子实体、孢子和菌丝体所提取的三萜类化合物组份(triterpenoid components)已被证实具有抗肿瘤、免疫调节等作用[1~4]。灵芝三萜类化合物的

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