Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2021-9-3-185-194

STANDARDIZATION PROBLEMS OF MEDICINAL PREPARATIONS FROM *RHODIOLA ROSEA* L.

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Received 15 Feb 2021 Accepted 16 May 2021

Rhodiola rosea L. rhizomes and roots are pharmacopoeial raw materials, which are used in official medicine for obtaining medicines with adaptogenic activity. One of the most common problems in the production of medicines from *Rhodiola rosea* L. rhizomes and roots is the use of poor quality medicinal plant materials, which leads to the absence of biologically significant compounds in the preparations. One of the possible reasons is the shortcomings in the existing approaches to the standardization of *Rhodiola rosea* L. raw materials and preparations.

The aim of the study is the improvement of approaches to the standardization of medicinal preparations from *Rhodiola rosea* L. rhizomes and roots.

Materials and methods. Experimental and industrial samples of liquid extract from *Rhodiola rosea* L. roots, as well as reference samples of rosavin and salidroside, were used as materials of the research. The HPLC analysis was carried out using a Milichrom-6 chromatograph (NPAO Nauchpribor) under the following conditions of reversed-phase chromatography in an isocratic mode: a steel column KAKH-6-80-4 (2 mm x 80 mm; Separon-C18 7 μm), a mobile phase – acetonitrile: 1% solution of acetic acid in water in the ratio of 14:86, the elution rate was 100 μL/min, the eluent volume was 2000 μL. The constituents were detected at the wavelength of 252 nm (rosavin) and 278 nm (salidroside).

Results. An assay of rosavin and salidroside in the liquid extract of *Rhodiola rosea* L. was developed using the HPLC method. It was determined that the content of rosavin in the samples of the liquid extracts obtained from *Rhodiola rosea* L. rhizomes and roots of the pharmacopoeial quality, varied from 0.21%±0.03% to 0.32%±0.04%, salidroside – from 1.13% ±0.05% to 2.71%±0.12%, respectively. The results of statistical processing indicate that the relative error of the average result for the determination of rosavin and salidroside in the preparations of *Rhodiola rose*a L. with a confidence level of 95% does not exceed ±6.0%.

Conclusion. Thus, methodological approaches to the analysis of medicinal preparations from *Rhodiola rosea* L. rhizomes and roots have been substantiated. These methodological approaches consist of the quantitative determination of the dominant and diagnostically significant biologically active compounds – rosavin and salidroside.

Keywords: *Rhodiola rosea* L.; rhizomes and roots; liquid extract; rosavin; salidroside; high performance liquid chromatography

Abbreviations: HPLC – high performance liquid chromatography; TLC – thin-layer chromatography; UV – ultraviolet; PM – pharmacopoeial monograph; GPM – general pharmacopoeial monograph; NMR – nuclear magnetic resonance

ВОПРОСЫ СТАНДАРТИЗАЦИИ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ РОДИОЛЫ РОЗОВОЙ

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Получено 15.02.2021 Принята к печати 16.05.2021

For citation: V.A. Kurkin, T.K. Ryazanova. Standardization problems of medicinal preparations from *Rhodiola rosea* L. *Pharmacy & Pharmacology.* 2021;9(3):185-194. **DOI:** 10.19163/2307-9266-2021-9-3-185-194

Для цитирования: В.А. Куркин, Т.К. Рязанова. Вопросы стандартизации лекарственных препаратов родиолы розовой. *Фармация и фармакология.* 2021;9(3):185-194. **DOI:** 10.19163/2307-9266-2021-9-3-185-194

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Родиола розовая (*Rhodiola rosea* L.), фармакопейным сырьем которой являются корневища и корни, применяется в официальной медицине для получения лекарственных препаратов с адаптогенной активностью. Одной из распространенных проблем при производстве лекарственных препаратов из корневищ и корней родиолы розовой является использование недоброкачественного лекарственного растительного сырья, что приводит к отсутствию значимых биологически активных соединений в препаратах. Одной из возможных причин являются недостатки в существующих подходах к стандартизации сырья и препаратов родиолы розовой.

Цель. Совершенствование существующих подходов к стандартизации лекарственных препаратов корневищ и корней родиолы розовой.

Материалы и методы. В качестве объектов исследования использовали экспериментальные и промышленные образцы экстракта жидкого корневищ и корней родиолы розовой, а также стандартные образцы розавина и салидрозида. ВЭЖХ-анализ осуществляли с использованием хроматографа «Милихром-6» (НПАО «Научприбор») в следующих условиях: метод – обращенно-фазовая хроматография в изократическом режиме (стальная колонка «КАХ-6-80-4», размер 2 мм х 80 мм; Сепарон-С18 7 мкм); подвижная фаза – ацетонитрил : 1% раствор уксусной кислоты в воде в соотношении 14:86; скорость элюирования – 100 мкл/мин; объем элюента – 2000 мкл. Детекцию веществ осуществляли при длине волны 252 нм (розавин) и 278 нм (салидрозид).

Результаты. С использованием метода ВЭЖХ разработана методика количественного определения розавина и салидрозида в жидком экстракте родиолы розовой. Определено, что содержание розавина в образцах жидких экстрактов, полученных из корневищ и корней родиолы розовой фармакопейного качества, варьирует от 0,21%±0,03% до 0,32%±0,04%; салидрозида – от 1,13%±0,05% до 2,71%±0,12% соответственно. Результаты статистической обработки свидетельствуют о том, что относительная ошибка среднего результата определения розавина и салидрозида в препаратах родиолы розовой с доверительной вероятностью 95% не превышает ±6,0%.

Заключение. Таким образом, в работе обосновываются методологические подходы к анализу лекарственных препаратов корневищ и корней родиолы розовой (*Rhodiola rosea* L.), заключающиеся в количественном определении доминирующих и диагностически значимых биологически активных соединений – розавина и салидрозида.

Ключевые слова: родиола розовая; *Rhodiola rosea* L.; корневища и корни; жидкий экстракт; розавин; салидрозид; высокоэффективная жидкостная хроматография

Список сокращений: ВЭЖХ – высокоэффективная жидкостная хроматография; ТСХ – тонкослойная хроматография; УФ – ультрафиолетовая область; ФС – фармакопейная статья; ОФС – общая фармакопейная статья; ЯМР – ядерный магнитный резонанс

INTRODUCTION

Rhodiola rosea L. rhizomes and roots are pharmacopoeial raw materials used in official medicine to obtain pharmaceuticals with adaptogenic activity [1–6]. The species of the *Rhodiola* L. genus have been used for a long time as adaptogens in Russia and northern Europe countries. Recently, a number of new pharmacological properties have also been detected in *Rhodiola rosea* L. preparations, i.e. antioxidant, anxiolytic, nootropic, antidepressant, and immunomodulatory activities [7–13]. It was reported that *Rhodiola rosea* L. preparations increase physical endurance, reduce fatigue and have a therapeutic effect in disorders of the gastrointestinal tract, cardiovascular system and central nervous system. Some studies have shown that *Rhodiola rosea* L. preparations inhibit the growth of malignant neoplasms. At the same time, the range of medicinal products on the basis of *Rhodiola rosea* L. rhizomes and roots, approved for use in the Russian Federation, is represented by only liquid extracts from different manufacturers[1]. *Rhodiola rosea* L. liquid extracts have been approved for use since 1975; they are recommended as an adaptogenic and tonic agent that is not inferior in its activity to ginseng [14].

Phytochemical studies have shown that the biological activity of *Rhodiola rosea* L. materials and preparations is due to six classes of compounds: phenylpropanoids, flavonoids, phenolic alcohols, phenolic acids, monoterpenes and sterols. The main biologically active compounds that determine the pharmacological activity of *Rhodiola rosea* L. raw materials and preparations are phenylpropanoids (rosavin, rosin, rosarin) and phenolic alcohols (tyrosol, salidroside) [15–20]. Phenylpropanoids are known for antioxidant, neurostimulating, adaptogenic activities. The adaptogenic activity of *Rhodiola rosea* L. raw materials and preparations, is also associated with the presence of phenolic alcohols.

In the State Pharmacopoeia of the Russian Federation of the XIV edition (PM.2.5.0036.15 “Rhodiola rosea L. rhizomes and roots” and PM.3.4.0008.18 “Rhodiola rosea L. rhizomes and roots extract liquid”), standardization of *Rhodiola rosea* L. raw materials and preparations provides for the quantitative determination of the salidroside content and the total amount of cinnamic alcohol glycosides calculated on rosavin[2,3] [21]. The analysis has been carried out by high performance liquid chromatography (HPLC) with an UV detection (at 219 nm – determination of salidroside, at 250 nm – determination of the total amount of cinnamic alcohol glycosides calculated on rosavin). The procedure has been carried out using a column 250×4.0 mm, endcapped octadecylsilyl (C18) silica gel for chromatography, 5 µm), for a mobile phase – acetonitrile: phosphate buffer (pH 7.0), the elution in a gradient mode with an increase of acetonitrile concentration from 11% to 60%, the elution rate

[1] The State Register of Medicines. Available from: http://grls.rosminzdrav.ru/grls.aspx.

[2] PM.2.5.0036.15 “Rhodiola rosea rhizomes and roots”

[3] PM.3.4.0008.18 “Rhodiola rosea rhizomes and roots extract liquid”

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DOI: 10.19163/2307-9266-2021-9-3-185-194

– 1.0 ml/min, the volume of the injected sample – 10 µl, the run time – 35 min.

At the same time, the expediency of the determination of the amount of cinnamic alcohol glycosides calculated on rosavin, causes doubt. Rosavin is the most labile compound, which, in comparison with other cinnamic alcohol glycosides, is more sensitive to the conditions for harvesting and storing raw materials due to the possibility of enzymatic destruction under the influence of the enzyme vicianosidase [1, 2]. Vicianosidase promotes the cleavage of vicianose, a carbohydrate fragment of the rosavin molecule, and exhibits a maximum activity in the temperature range of 40–60°C, which had been previously recommended for *Rhodiola rosea* L. raw materials drying. Storage of undried rhizomes, extraction of fresh raw materials with ethanol at room temperature also contribute to the destructive effect of enzymes on the composition of biologically active components. The destruction of rosavin leads to the formation of biologically inactive cinnamic alcohol, and accordingly, the pharmacological activity of *Rhodiola rosea* L. raw materials and preparations decreases [1, 2].

In this regard, in order to standardize the plant raw materials, a more conceptually correct approach, in the authors' opinion, is to quantify not the total cinnamic alcohol glycosides, but the most labile component – rosavin. The level of its content reliably reflects the correct storage and drying conditions for *Rhodiola rosea* L. rhizomes and roots [1, 2].

These assumptions are confirmed by the results of the selective quality control of *Rhodiola rosea* L. raw materials and preparations (extracts, granular powders) [22–26]. Booker A. et al. have analyzed 40 commercial *Rhodiola rosea* L. products from various suppliers on the European Union market and have found that approximately one fifth of these products did not contain rosavin, one of the main components of *Rhodiola rosea* L. Moreover, some products did not contain salidroside, the component typical of *Rhodiola* sp. In about 80% of the remaining commercial products, the rosavin content was lower than declared, and it was assumed that they had been obtained from other species of the *Rhodiola* genus [23].

Therefore, rosavin is precisely the marker that makes it possible to reliably assess the quality of *Rhodiola rosea* L. raw materials and preparations, and the problem of a proper quality control of *Rhodiola rosea* L. raw materials and preparations is of a worldwide meaning.

It should be also notified that the quantitative determination methods included in the monographs, provide for the HPLC analysis in the gradient elution mode [21]. It is known that when using the gradient mode, the correction of conditions is more critical, it can lead to incorrect identification of peaks, their overlap or shifts, at which the analytes can leave before or after the specified time of the chromatogram registration. In the authors' opinion, the selection of conditions for the analysis in the isocratic mode will increase the reproducibility of the technique[4] [21]. In addition, the 219 nm wavelength used in the pharmacopoeial method for the detection of salidroside is less specific with respect to the accompanying components in comparison with the other absorption maximum of this compound – 278 nm [2].

THE AIM of the study was to improve the existing approaches to the standardization of medicinal products of *Rhodiola rosea* L. raw materials and preparations, adopted in the State Pharmacopoeia of the Russian Federation of the XIV edition.

MATERIALS AND METHODS

Research materials

Experimental and industrial samples of the liquid extract of *Rhodiola rosea* L. rhizomes and roots were used as research materials. The experimental samples were obtained by the method of modified maceration from the medicinal plant materials, harvested in 2016–2018 (Altai region). A reference sample of rosavin that meets the requirements of PM 42-0071-01, was obtained by the authors of the article from *Rhodiola rosea* L. rhizomes and roots using silica gel column chromatography and subsequent recrystallization from 95% ethyl alcohol.

A reference sample of salidroside was obtained by the authors from *Rhodiola rosea* L. rhizomes and roots using silica gel column chromatography, rechromatography on polyamide and subsequent recrystallization from a mixture of chloroform and 95% ethyl alcohol. It was identified by means of TLC, UV-, NMR-spectroscopy and had a melting point of 162–164°C, the purity not less than 98.0% and corresponded to the requirements of the PM draft.

Acetonitrile (ZAO "Component-reagent", "For high performance liquid chromatography"); the water obtained while using a system for obtaining deionized water by a multistage purification system (adsorption, reverse osmosis, membrane filtration) and checked for purity under the conditions of chromatographic analysis, were used in the work. The rest of the reagents were of analytical reagent grade or of chemically pure grade.

Preparation of a salidroside standard sample solution. About 0.025 g (accurately weighed) of the state standard sample of salidroside (the content of the main substance ≥98%) is placed in a volumetric flask with a capacity of 50 ml, dissolved in a small amount of 95% ethanol, brought to the mark with ethanol 95%, and mixed.

Preparation of rosavin standard solution. About 0.025 g (accurately weighed) of the state standard sample of rosavin (content of the main substance ≥98%) is placed in a volumetric flask with a capacity of 50 ml, dissolved in a small amount of 95% ethanol when heated in a boiling water bath, brought to the mark with ethanol 95%, mixed.

[4] GPM.1.2.1.2.0001.15 Chromatography.

ISSN 2307-9266 e-ISSN 2413-2241
Scientific and Practical Journal
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Conditions for chromatographic separation

HPLC analysis was carried out using a Milichrom-6 chromatograph (NPAO Nauchpribor) under the following conditions of reverse-phase chromatography in an isocratic mode: steel column KAH-6-80-4 (2 mm×80 mm; Separon-C18, 7 µm), a mobile phase – acetonitrile: a 1% solution of acetic acid in water in the ratio of 14:86, the elution rate was 100 µL/min, the eluent volume was 2000 µL. The compounds were detected at the wavelength of 252 nm (rosavin) and 278 nm (salidroside). The volumes of the injected samples were: 3 µl (the reference solutions of rosavin and salidroside) and 5 µl (the experimental samples of *Rhodiola rosea* L. liquid extracts).

System suitability assessment

The suitability of the chromatographic system was evaluated in accordance with the General Pharmacopoeia Monograph 1.2.1.2.0001.15 "Chromatography". The indicators of the chromatographic system suitability (column efficiency, resolution between peaks, asymmetry factor) were calculated based on the results of a 5-fold analysis of 5 µl of the *Rhodiola rosea* L. rhizomes and roots liquid extract solution.

The results of evaluating the suitability of the system confirm the suitability of the chromatographic system for the quantitative determination of salidroside and rosavin in *Rhodiola rosea* L. raw materials and preparations (Table 1).

Methods for the simultaneous quantitative determination of rosavin and salidroside in Rhodiola rosea L. rhizomes and roots liquid extracts

1 ml of *Rhodiola rosea* L. rhizomes and roots liquid extract is placed in a 25 ml volumetric flask, diluted to the mark with purified water. Before the analysis, an aliquot of the sample is additionally filtered through a Milipore membrane filter (0.45 µm) (the test solution).

5 µl of the test solution is injected into a Milichrom-6 liquid chromatograph with an UV detector. Chromatography is carried out under the conditions of reverse phase chromatography in an isocratic mode: steel column "KAH-6-80-4" (2 mm x 80 mm; Separon-C18, 7 µm), a mobile phase – acetonitrile: a 1% solution of acetic acid in water in the ratio of 14:86, the elution rate was 100 µl/min, the eluent volume was 2000 µl.

The compounds were detected at the wavelengths of 252 nm (rosavin) and 278 nm (salidroside).

In parallel, 3 µl of solutions of reference salidroside and rosavin samples are injected into the chromatograph and chromatographed as described above. The height of the salidroside peak on the chromatogram is determined at the wavelength of 278 nm and the area of the rosavin peak on the chromatogram at the wavelength of 252 nm. The average values based on the results of three parallel determinations, are calculated.

The salidroside content (X in percent) is calculated by the formula:

$$X, \% = \frac{H_i \times m_{st} \times V_1 \times V \times 100}{H_{st} \times V_{st} \times V_2 \times V_{al}},$$

where: H_i is the height of the salidroside peak on the test solution chromatogram, absorbance units; H_{st} is the height of the salidroside peak in the reference solution chromatogram, absorbance units; m_{st} is the exact weight of salidroside reference sample, g; V_{st} is the volume of the prepared salidroside reference solution, ml; V_1 is the volume of the injected sample of the reference solution, µl; V_2 is the volume of the injected test solution, µl; V is the volume of a volumetric flask in which an aliquot of *Rhodiola rosea* L. rhizomes and roots liquid extracts was diluted, ml; V_{al} is the volume of an aliquot of *Rhodiola rosea* L. rhizomes and roots liquid extracts, ml.

The content of rosavin (X in percent) is calculated by the formula:

$$X, \% = \frac{S_i \times m_{st} \times V_1 \times V \times 100}{S_{st} \times V_{st} \times V_2 \times V_{al}},$$

where: S_i is the area of the rosavin peak on the test solution chromatogram; S_{st} is the area of the rosavin peak in the reference solution chromatogram; m_{st} is the exact weight of rosavin reference sample, g; V_{st} is the volume of the prepared rosavin reference solution, ml; V_1 is the volume of the injected sample of the reference solution, µl; V_2 is the volume of the injected test solution, µl; V is the volume of a volumetric flask in which an aliquot of *Rhodiola rosea* L. rhizomes and roots liquid extracts was diluted, ml; V_{al} is the volume of an aliquot of *Rhodiola rosea* L. rhizomes and roots liquid extracts, ml.

Validation of methods

The validation assessment of the developed methodology was carried out according to the following indicators: specificity, linearity, accuracy (recovery), precision. The specificity of the methods was determined by the correspondence of retention times of the salidroside and rosavin peaks on the HPLC chromatograms of the reference solutions and the peaks corresponding to these standards on the HPLC chromatogram of the test solution, as well as by the resolution between the closest peaks and the asymmetry factor of the peaks of salidroside and rosavin.

The determination of linearity was carried out at five concentration levels of reference sample solutions (with concentrations ranging from 0.1467 to 1.4667 mg/ml for salidroside and from 0.1200 to 0.9600 mg/ml for rosavin). Based on the data obtained, a graph was built in the coordinates "concentration, mg/ml – peak height" or "concentration, mg/ml – peak area" and there were calculated the linear regression equation ($Y = ax + b$), the value of the coefficient of the determination (r^2), a standard deviation using Microsoft Excel 2013 software.

The accuracy of the method was tested by introducing an exact amount of reference samples of rosavin and salidroside in the range of 80% to 120% of the initial content, into the aliquot of *Rhodiola rosea* L. preparation.

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Table 1 – Determination of the chromatographic system suitability

Chromatographic system parameter	Value	Limit
Column efficiency (salidroside)	5.100	At least 5000 theoretical plates
Column efficiency (rosavin)	5.201	
Resolution between closest peaks (salidroside)	1.6	Not less than 1.5
Resolution between closest peaks (rosavin)	2.3	
Asymmetry factor (salidroside)	1.47	Not more than 1.5
Asymmetry factor (rosavin)	1.21	

Table 2 – Metrological characteristics of the method for the quantitative determination of salidroside and rosavin in *Rhodiola rosea* L. liquid extracts

Analyte	f	$\overline{X}$,%	S	P,%	t (P,f)	$\Delta\overline{X}$	$\overline{\varepsilon}$,%
Salidroside	10	2.02	0.160114	95	2.23	±0.11	±5.33
Rosavin	10	0.22	0.014460	95	2.23	±0.04	±4.43

Note: f – degrees of freedom; $\overline{X}$ – average; S – standard deviation; P – confidential probability; t – Student's t-test; $\Delta\overline{X}$ – half-width of the confidence interval of the mean result; $\overline{\varepsilon}$ – mean relative error.

Table 3 – Results of determining the accuracy of the analytical procedure (salidroside)

Initial content of salidroside, mg in 1 ml of water-alcohol extract	Added salidroside mg/ml	Salidroside content, mg/ml		Error	
		Estimated	Found	Absolute, mg/ml	Relative,%
20.18	16.00	36.18	37.00	0.82	2.27
20.18	20.00	40.18	39.50	–0.68	–1.69
20.18	25.00	45.18	45.00	–0.18	–0.40

Table 4 – Results of determining the accuracy of the analytical procedure (rosavin)

Initial content of rosavin, mg in 1 ml of water-alcohol extract	Added rosavin mg/ml	Rosavin content, mg/ml		Error	
		Estimated	Found	Absolute, mg/ml	Relative,%
2.19	1.75	3.94	4.00	0.06	1.52
2.19	2.20	4.39	4.25	–0.14	–3.19
2.19	2.60	4.79	4.61	–0.18	–3.76

Table 5 – The content of rosavin and salidroside in experimental and industrial samples of liquid extracts from *Rhodiola rosea* L. rhizomes and roots

No.	Sample	Salidroside content,%	Rosavin content,%
1.	Experimental sample No. 1 (obtained from raw materials harvested in 2016)	2.13% ±0.05%	0.21% ±0.03%
2.	Experimental sample No. 2 (obtained from raw materials harvested in 2018)	2.71% ±0.12%	0.32% ±0.04%
3.	Industrial sample No. 1	1.62% ± 0.05%	Not found
4.	Industrial sample No. 2	2.75% ± 0.08%	Not found
5.	Industrial sample No. 3	2.55% ± 0.07%	Not found
6.	Industrial sample No. 4	1.20% ± 0.04%	Not found
7.	Industrial sample No. 5	1.12% ± 0.06%	Not found
8.	Industrial sample No. 6	0.96% ± 0.04%	Not found

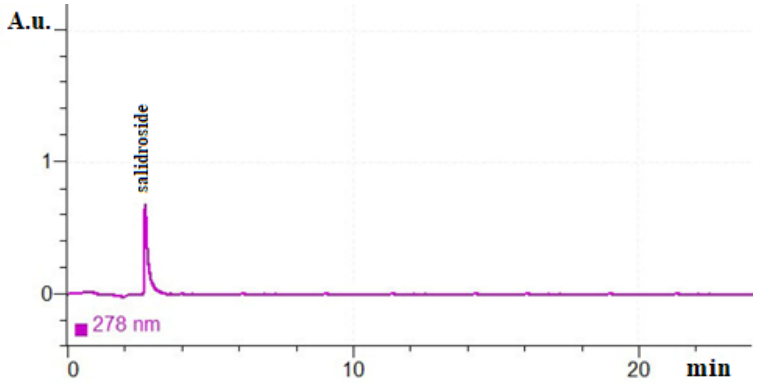

Figure 1 – HPLC chromatogram of salidroside reference sample solution, 0.88 mg/ml
Note: detection at the wavelength of 278 nm

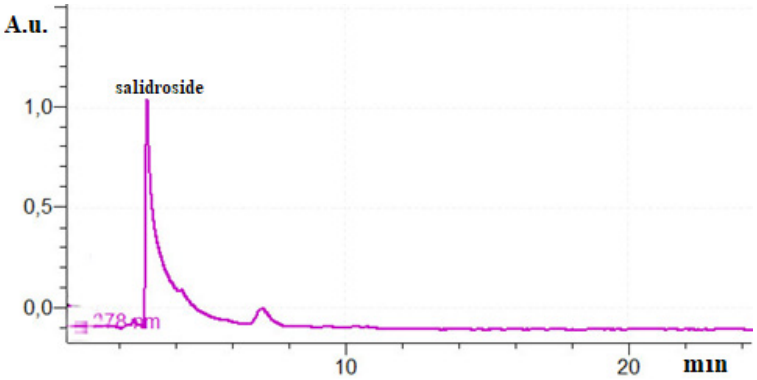

Figure 2 – HPLC chromatogram of the experimental sample of liquid extract from *Rhodiola rosea* L. rhizomes and roots
Note: detection at the wavelength of 278 nm

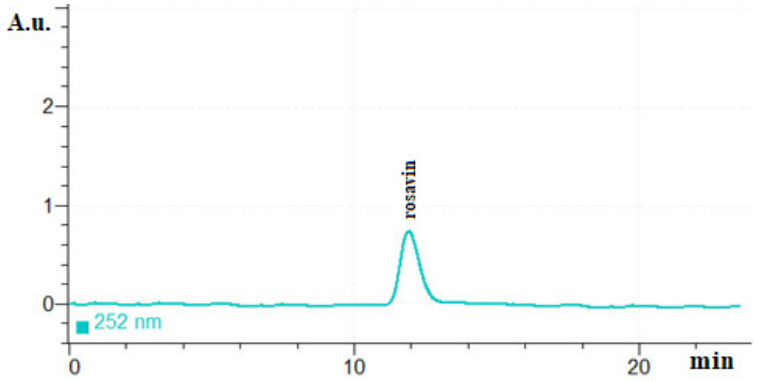

Figure 3 – HPLC chromatogram of rosavin reference sample solution, 0.60 mg/ml
Note: detection at the wavelength of 252 nm

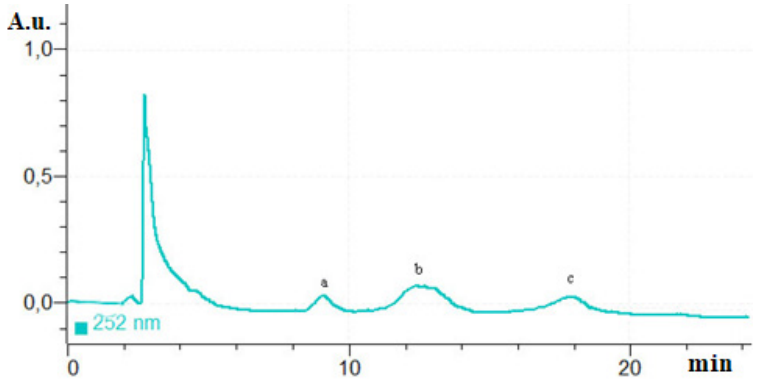

Figure 4 – HPLC chromatogram of the experimental sample of liquid extract from *Rhodiola rosea* L. rhizomes and roots
Note: a – rosarin; b – rosavin; c – rosin; detection at the wavelength of 252 nm

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DOI: 10.19163/2307-9266-2021-9-3-185-194

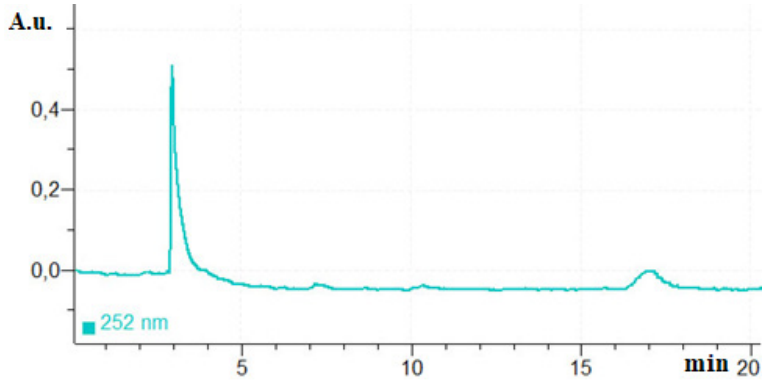

Figure 5 – Representative HPLC chromatogram of industrial samples of liquid extract from *Rhodiola rosea* L. rhizomes and roots
Note: detection at the wavelength of 252 nm

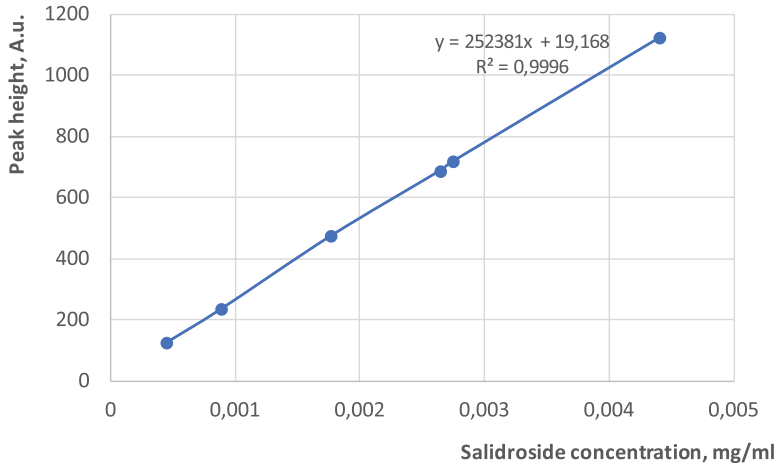

Figure 6 – Graph of the dependence of the peak height on the concentration of salidroside in the sample and the linear regression equation

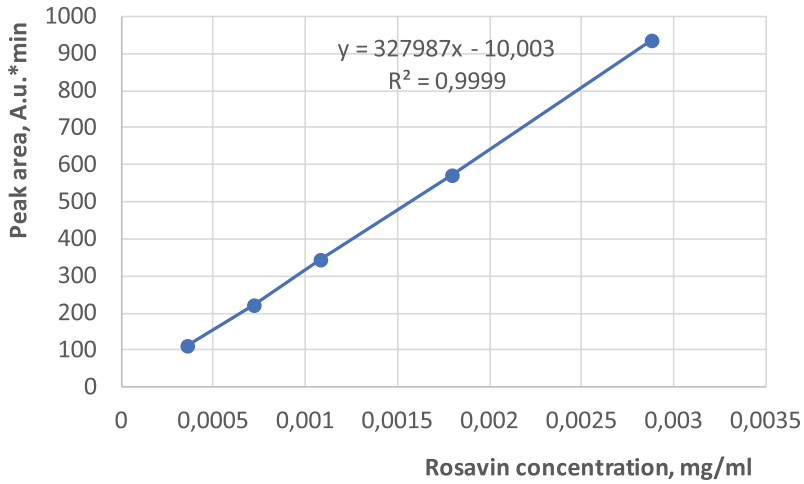

Figure 7 – Graph of the dependence of the peak area on the concentration of rosavin in the sample and the linear regression equation

RESULTS AND DISCUSSION

At the preliminary stage, under the conditions of chromatographic separation described above, the content of rosavin and salidroside in *Rhodiola rosea* L. rhizomes and roots used to obtain experimental samples of the liquid extract, was analyzed. It was determined that the content of rosavin in *Rhodiola rosea* L. rhizomes and roots varied from 1.17%±0.04% to 1.41% ± 0.06% and salidroside – from 1.63%±0.05% to 2.88%±0.12%, respectively. In the sample harvested in 2020, rosavin was not detected, although other glycosides of cinnamic alcohol were present, which indicates improper storage conditions of the raw materials.

Under the proposed conditions of HPLC analysis, the retention times of salidroside peaks on the chromatograms of the salidroside reference solution, the aqueous-alcoholic extracts from *Rhodiola rosea* L. raw materials and the solutions obtained as a result of dilution of the experimental samples of *Rhodiola rosea* L. liquid extracts, were (2.780 ± 0.077), (2.979 ± 0.070) and (2.790±0.087) min, respectively (Fig. 1 and 2). For rosavin, the corresponding values were (12.424±0.080), (12.824±0.070) and (12.429±0.070) min (Fig. 3 and 4). Rosavin was not detected in any of the 6 analyzed industrial samples.

The dependence of the height and area of the chromatographic peak on the salidroside concentration was described by a linear regression model in the concentration range from 0.1467 to 1.4667 mg/ml (Fig. 6). However, the correlation coefficient for the dependence of the peak height on the salidroside concentration was 0.9996, for the dependence of the peak area from the concentration it was 0.9888. In this regard, the calculation of the salidroside content in the test samples was carried out using the peak height.

For the dependence of the height and area of the peak on the concentration of rosavin in the concentration range from 0.1200 to 0.9600 mg/ml, the correlation coefficients were 0.9973 and 0.9999 (Fig. 7). Therefore, the determination of the content of rosavin was carried out using the peak area.

The indicated concentration ranges of salidroside and rosavin can be considered as the range of the analytical procedures.

The metrological characteristics of the proposed HPLC procedure indicate that the error in determining the average result of the salidroside content in the liquid extract of *Rhodiola rosea* L. rhizomes and roots with a confidence level of 95% is ±5.33%, rosavin – ±4.43% (Table 2). The accuracy of the method was determined by adding solutions of salidroside and rosavin with the known concentration (80%, 100%, and 120%) to the aliquot of the experimental drug. At the same time, the average percentage of the recovery was 100.06% and 98.19%, respectively (Tables 3 and 4). The errors in the determination of salidroside and rosavin in the samples with additives of the reference samples were within the error of a single determination, which indicates the absence of a systematic error.

The study of the technique repeatability indicates the convergence of the obtained concentrations of salidroside and rosavin: the relative error of the average result of determining the content of salidroside in *Rhodiola rosea* L. rhizomes and roots with a confidence level of 95% is ±5.61% and ±4.70%, respectively. The one of a single determination is ±17,7% and ±14.7%, respectively. When evaluating the intra-laboratory precision, satisfactory results were also shown, since the relative error in the determination of rosavin and salidroside on the first and second days of the analysis was in the range from 0.90 to 1.09.

It was determined that the content of rosavin in experimental samples of the liquid extracts obtained from *Rhodiola rosea* L. rhizomes and roots of the pharmacopoeial quality varies from 0.21%±0.03% to 0.32%±0.04% and the one of salidroside – from 2.13%±0.05% to 2.71%±0.12%, respectively (Table 5). In the analyzed industrial samples of two Russian manufacturers, the salidroside content varied from 0.96%±0.04% to 2.75%±0.08%. Rosavin was not found in any of the samples tested.

Therefore, the absence of rosavin in the preparations of *Rhodiola rosea* L., according to our data and other published results [22–26], is a common problem. Possible reasons are the use of other species of the *Rhodiola* L. genus for the preparation of drugs or improper conditions for harvesting, drying and storage of the medicinal plant raw materials, as well as their processing.

It is known that salidroside is present in almost all types of *Rhodiola* sp., it is not subject to enzymatic or thermal degradation, and its content in rhizomes and roots does not depend on its habitat [27]. Rosavin, unlike salidroside and other phenylpropanoids, is found only in *Rhodiola rosea* L. rhizomes, and it is the most labile of its components, since it is subject to the selective enzymatic degradation. Drying the rhizomes of this plant at the temperature of 50–60°C leads to the greatest selective enzymatic degradation of rosavin to aglycone – cinnamic alcohol. The temperature range of 70–80°C is recommended as the optimal drying conditions for *Rhodiola rosea* L. rhizomes [1].

The variability of the rosavin content depending on its habitat, was revealed. The maximum content of rosavin and salidroside was notified in *Rhodiola rosea* L. rhizomes of the Altai origin [27]. For the rhizomes of the Mongolian population of *Rhodiola rosea* L., the peculiarity of its chemical composition is a high content of flavonoids (approximately 200 times more than in other samples of the raw materials), especially in herbacetin derivatives [28].

It was also determined that rosavin appears in the rhizomes of the plant only in the second year of life and reaches its maximum value in the fourth year of life. Salidroside begins to accumulate in plants in the first year of life, reaching its maximum, just as in the case of rosavin, in the fourth year of life [2]. Taking into account the fact that the phytomass of *Rhodiola rosea* L. rhizomes actively grows in the 5th and 6th years of plant life against the background of maintaining a high content of rosavin, recommendations for harvesting the raw materials for 5–6-year-old plants are justified [2].

Therefore, the presence of rosavin is a reliable indicator of a good quality of *Rhodiola rosea* L. raw materials and preparations.

Taking into account the obtained data, it is possible to recommend a lower limit of the rosavin content in *Rhodiola rosea* L. liquid extracts – 0.1%, salidroside – 0.8%.

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CONCLUSION

Thus, the article proposes HPLC procedures for the simultaneous assays of the two most significant biologically active compounds of *Rhodiola rosea* L. rhizomes and roots in medicines obtained on their basis under the conditions of an isocratic elution mode. The error in determining the average result of the rosavin and salidroside content in the raw material of *Rhodiola rosea* L. did not exceed 6.0%.

Methodological approaches to the development of procedures for the pharmacopoeial analysis of *Rhodiola rosea* L. preparations have been considered. The quality of medicinal preparations from *Rhodiola rosea* L. rhizomes and roots is directly related to the quality of medicinal plant raw materials. Herewith, the most labile biologically active component, which is the most susceptible to the enzymatic degradation when the conditions for drying, storage of raw materials and their processing are violated, is rosavin. In this regard, it is this phenylpropanoid that is the marker of the quality of *Rhodiola rosea* L. raw materials and preparations which should be first of all paid attention to.

FUNDING

The study was carried out within the framework of the project "Development of national approaches to the standardization of herbal medicinal products, medicinal plant raw materials and phytobiotechnological products" with financial state support in the form of a Scholarship of the President of the Russian Federation for young scientists and postgraduates carrying out promising research and development in priority areas of modernization of the Russian economy".

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

V.A. Kurkin – planning of the study, participation in the development of the concept and design of the study, final approval of the manuscript for publication, processing the results obtained, verification of critical intellectual content; T.K. Ryazanova – data collecting, experiment conducting, analyzing and interpreting the data obtained, preparing a manuscript draft, literature analyzing, manuscript writing.

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