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Quantitative determination of total flavonoids in *Glycyrrhiza Glabra* L. herbs

O.A. Belova, V.A. Kurkin, M.V. Egorov

Samara State Medical University,
89, Chapaevskaya Str., Samara, Russia, 443099

E-mail: v.a.kurkin@samsmu.ru

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Licorice herb (*Glycyrrhiza glabra* L.) is a promising herbal raw material, which can be comprehensively used to develop drugs with an anti-inflammatory action.

The aim of the article was to development a quantitative determination method of total flavonoids in *Glycyrrhiza glabra* L. herbs.

Materials and methods. The subjects of research were 5 samples of licorice herb harvested in summer in various places of growing and cultivation. Pinostrobin was used as a standard sample. The registration of the electronic spectra was carried out with a spectrophotometer (Analytik Jena AG, Germany) by differential spectrophotometry, 96% ethanol was used as a solvent.

Results. The methods for quantitative determination of total flavonoids in *Glycyrrhiza glabra* L. was carried out at an analytical wavelength of 310 nm equivalent to pinocembrin. The optimum parameters for the extraction of total flavonoids from *Glycyrrhiza glabra* L. were as follows: the extractant – 90% ethanol; the «raw material-extractant» ratio was 1:50; the extraction time was 60 min; the degree of atomization was 2 mm. The content of total flavonoids for the *Glyccyrhiza glabra* L. herb has been determined, it varies from 0.39±0.002 to 3.41±0.015% with the humidity of the vegetative raw material from 9.97±0.003 to 10.03±0.003% depending on the place of the vegetation, cultivation and year of the raw material collection. The error of the single determination with a 95% confidence level was ±0.73.

Conclusion. The developed methods for the quantitative determination of total flavonoids in *Glycyrrhiza glabra* L. herbs can be used to solve the issues of standardization of these medicinal plant raw materials.

Keywords: licorice; *Glycyrrhiza glabra* L.; herb; flavonoids; pinocembrin; standardization; spectrophotometry

Abbreviations: SS – standard sample; PhM – pharmacopoeial monograph; GPhMK – general pharmacopoeial monograph; BAC – biological active compounds; HPLC – High Performance Liquid Chromatography.

Методика количественного определения суммы флавоноидов в траве солодки голой

О.А. Белова, В.А. Куркин, М.В. Егоров

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Самарский государственный медицинский университет»
Министерства здравоохранения Российской Федерации,
443099, Россия, г. Самара, ул. Чапаевская, д. 89

E-mail: v.a.kurkin@samsmu.ru

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Трава солодки голой (*Glycyrrhiza glabra* L.) является перспективным растительным сырьём, которое может быть комплексно использовано для разработки лекарственных препаратов с противовоспалительным действием.

Цель. Разработка методики количественного определения суммы флавоноидов в траве солодки голой.

Материал и методы. Объектами исследования являлись 5 образцов травы солодки голой, заготовленных в летний период времени в различных местах произрастания и культивирования. В качестве стандартного образца использовали пиностробин. Регистрацию УФ-спектров проводили с помощью спектрофотометра «Specord 40»

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(Analytik Jena AG, Германия) методом дифференциальной спектрофотометрии. В качестве растворителя использовали спирт этиловый 96%.

Результаты. Количественное определение суммы флавоноидов в траве солодки голой проводили при аналитической длине волны 310 нм в пересчёте на пиноцембрин. Установлены оптимальные параметры экстрагирования суммы флавоноидов из травы солодки голой: экстрагент – спирт этиловый 90%; соотношение «сырье-экстрагент» – 1:50; время экстракции – 60 мин; степень измельчения сырья – 2 мм. Определено содержание суммы флавоноидов для травы солодки голой, которое варьирует от 0,39±0,002 до 3,41±0,015% с учётом влажности растительного сырья от 9,97±0,003 до 10,03±0,003% в зависимости от места произрастания, культивирования и года сбора растительного сырья. Погрешность единичного определения с доверительной вероятностью 95% составляла ±0,73%.

Заключение. Разработанная методика количественного определения флавоноидов в траве солодки голой может быть использована для решения вопросов стандартизации указанного лекарственного растительного сырья.

Ключевые слова: солодка голая; *Glycyrrhiza glabra* L.; трава; флавоноиды; пиноцембрин; стандартизация; спектрофотометрия

Список сокращений: ЛП – лекарственный препарат; СО – стандартный образец; ФС – фармакопейная статья; БАВ – биологически активные вещества; ВЭЖХ – высокоэффективная жидкостная хроматография.

INTRODUCTION

Glycyrrhiza glabra L. (*Fabaceae* family) is one of the most researched and well-studied plants in the world. This is a perennial herbaceous plant with erect and sparsely branched stems, reaching a height of 50–100 cm (in exceptional cases up to 200 cm). The underground organs are well developed; its roots penetrate into the soil to a depth of 6–8 m and form a thick network, which helps maintain the licorice population. The leaves are alternate, compound, unpaired. The flowers are aggregated in loose racemes on long pedicels. The plant is common in many regions, even though it belongs to the Mediterranean species. In the valleys of the major rivers of Central Asia, Uzbekistan and Kazakhstan, licorice forms thickets[1,2].

The roots of this plant are widely used in officinal medicine. On the basis of isolated biologically active compounds (BAC) from the roots, many multicomponent drugs have been developed. In the course of studies, a group of scientists from Samara State Medical University and the All-Russian Institute of Medicinal and Aromatic Plants have also developed the state standard sample (SS) of glycyram (PhM-42-0034-00), with the specification of chemical structure, and studied physical and chemical properties of glycyrrhizic acid [1–4].

Licorice roots are valuable pharmacopoeial raw materials that are widely used for the production of drugs with an expectorant effect and an anti-inflammatory activity. This raw material is particularly popular not only in Russia but also abroad [5–7]. In recent decades, scientists have paid attention to various species of the genus *Glycyrrhiza* L. as promising sources of BACs used to create phytopreparations. Along with licorice roots, one of the promising sources is also the aerial part, i.e., the licorice herb [8, 9].

According to literature data, it is known that the above-ground part of licorice contains flavonoids, polysaccharides, tannins, triterpenoids, vitamins, etc. It is known that the flavonoid composition of licorice herb is represented by pinocembrin, glabranin, prunetin, astragalin and vitexin [9]. It should be concluded that the above-ground part of licorice is rich in a high content of BAC, among which flavonoids are of the greatest interest in terms of creating medicines.

Flavanones prevail in the herb of this plant. The dominant among them is pinocembrin (5,7- dihydroxyflavanone) the efficacy of which has been proven in preclinical practice. A number of *in vitro* and *in vivo* studies have shown that pinocembrin improved the regional cerebral blood flow of non-pedigreed rats and reduced postischemic neurovascular block damage. It follows that pinocembrin has a neuroprotective activity [10–14]. It has also a powerful antifibrotic effect, which explains its antioxidant properties. Pinocembrin alleviated bleomycin-induced skin fibrosis and a fibrosis-related protein expression in keloid tissues in xenografted mice [15, 16].

Pinocembrin had a protective effect against gentamicin-induced nephrotoxicity, which may be partially related to its antioxidant and anti-apoptotic effects, subsequently leading to the improved renal function [17]. This flavanone showed a pronounced

[1] Budantsev AL. Plant resources of Russia. Wild flowering plants, their component composition and biological activity. Vol. 3: *Fabaceae – Apiaceae*: A.L. Budantsev ed. Moscow: Association of Scientific Editions KMK; 2009. 599 p. Russian

[2] Maevsky PF. Flora of the middle belt of the European part of Russia. 11th ed. M.: KMC Scientific Publishers Association; 2014. 635 p. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2023-11-2-127-136

activity against Gram-positive bacteria, while its methyl ester pinostrobin (5-hydroxy-7-methoxyflavanone) was less pronounced against *Esherichia coli* due to its chemical structure determining the lipophilic properties of the substance and its affinity to the lipid membrane of gram-negative bacteria [18–20]. In an *in vitro* study, pinocembrin showed a photoprotective efficacy [20, 21] and inhibited an allergic airway inflammation [22].

In one study, pinocembrin showed a more pronounced anti-inflammatory activity than the non-selective cyclooxygenase inhibitor indomethacin. At the same time, the anti-inflammatory drugs of comparison were amidopyrine, hydrocortisone, and indomethacin. A formalin-induced inflammation in mice was reduced by 40.3% at the pinocembrin dose of 25 mg/kg and by 43.4% at the 50 mg/kg dose. In methylation and ethylation of pinocembrin, at position C_8, the formation of its derivatives led to a decrease in the anti-inflammatory activity [23].

In view of the above, a wide range of therapeutic uses of the licorice herb seems appropriate from the position of complex processing of raw materials to study the properties of water-alcoholic extracts and preparations based on the herb of this plant. This will expand the range of ideas about the pharmacological activity of flavonoids and substances of the above-ground part of *G. glabra* and evaluate the possibility of using this object in the creation of domestic drugs.

Based on the sum of flavonoids from the above-ground part of licorice, foreign scientists developed a drug called “Glacembrine” with anti-inflammatory and analgesic effects [24]. Glacembrin has undergone a number of clinical trials according to the following parameters: an acute toxicity, an anti-inflammatory activity. The results showed that “Glacembrin” did not differ from the similar indicator of pinocembrin, and the anti-inflammatory activity of the drug was higher than in the case of pinocembrin. In addition, “Glacembrin” has a pain-relieving effect on the model of acetic cortex in mice [23].

All of the above-mentioned has opened new opportunities for the use of the licorice herb in the pharmaceutical industry as a raw material for the manufacture of standardized drugs with anti-inflammatory and other effects. In general, the literary analysis showed an insufficient degree of study and elaboration of the licorice herb standardization. Attention should be paid to the developed methods of the quantitative determination of total flavonoids in the above- ground part of licorice by direct spectrophotometry equivalent to pinocembrin, proposed by Uzbek scientists [25]. In our opinion, this technique can give overestimated results of determination since other phenolic compounds also contribute to the optical density at the analytical wavelength of 290 nm. In addition, a multiple extraction (3 times) of raw materials cannot be always justified, because these extraction conditions can increase the error of the analysis method.

In this regard, the research is relevant in terms of improving the methods for a quantitative determination of total flavonoids in the *Glycyrrhiza glabra* L. herb.

THE AIM of the article was to work out methods for quantitative determination of total flavonoids in *Glycyrrhiza glabra* L.

MATERIALS AND METHODS

The objects of the study were five samples of the *Glycyrrhiza glabra* L. herb. They were prepared: No. 1 – in the Samara region (Kinelsky district, Alekseevka village), August 2021; No. 2 – in Samara city, the Botanical Garden of Samara State Medical University, August 2021; No. 3 – in the Orenburg region, (Sakmarsky district, Tatarskaya Kargala village), July 2017; No. 4 – in the Republic of Kazakhstan, Derzhavinsk city, June 2018; No. 5 – in the Samara region (Bolshechernigovsky district, Bolshaya Chernigovka village), August 2019. The moisture content of the plant raw materials was determined in accordance with the requirements of the Russian State Pharmacopoeia XIV edition GPhM.1.5.3.0007.15 «Determination of moisture content of medicinal plant raw materials and medicinal plant preparations»[3].

The used methods of spectrophotometry was performed in accordance with the requirements of the Russian State Pharmacopoeia XIV edition GPhM.1.2.1.1.0003.15 «Spectrophotometry in ultraviolet and visible regions». Spectral characteristics of aqueous-alcohol extracts were evaluated on a spectrophotometer «Specord 40» (Analytik Jena AG, Germany) in cuvettes with a layer thickness of 10 mm, 96% ethanol was used as a solvent.

The solution of pinostrobin prepared in 96% ethanol was used as a SS (Fig. 1). Pinostrobin standard

[3] Russian State Pharmacopoeia. XIV ed. Vol. 1–4. Moscow; 2018. Available from: http://http://femb.ru/femb/ pharmacopea.php

sample corresponded to the requirements of the pharmacopoeial article (PhM 42-0073-01) and was provided to the Scientific and Educational Center «Pharmacy» of Samara State Medical University to determine by high-performance liquid chromatography (HPLC) the degree of purity, which was not less than 98.0%. Aqueous-alcoholic extracts were prepared using 96% ethanol (brand name: chemical clean, Hippocrates, Russia). The necessary concentrations of alcohol (50, 60, 70, 80, 90%) were obtained by diluting 96% ethanol according to Table 5 of Appendix to the Russian State Pharmacopoeia XIV edition.

Preparation of working solutions for analysis by UV-spectrophotometry

The analytical sample of raw materials was crushed to the particle sizes, passing through a sieve with holes of 2 mm in diameter. About 1 g of the crushed raw material (exact weight) was placed in a conical heat-resistant Erlenmeyer flask with a 100 ml slit, 50 ml of 90% ethanol was added. The flask was closed with a stopper and weighed on Sarto GOSM laboratory scales (Russia) with an accuracy of ±0,001. The flask was attached to a reflux condenser and heated in a boiling water bath (moderate boiling) for 60 min. Then it was cooled for 30 min, closed with the same cap, weighed again and the missing extractant was replenished to the original volume. The extraction was filtered through a paper filter (red band).

To prepare test solution pinostrobin for the UV-spectrophotometry, about 0.02 g (exact weight) of the substance was weighed, placed in a 50 ml volumetric flask, dissolved in 30 ml of 96% ethanol and heated in a water bath. Use of 96% ethanol provided the best dissolution of the reference standard of pinostrobin. After cooling the contents of the flask to room temperature, its volume was brought to the mark with 96% ethanol (pinostrobin standard solution A). Then 1 ml of pinostrobin standard solution A was placed into a 25 ml volumetric flask, 2 ml of aluminum trichloride 3% alcohol solution was added and the solution volume was brought to the mark with 96% ethanol (test solution B of pinostrobin).

The reference solution was prepared as follows: 1 ml of pinostrobin A solution was placed in a 25 ml volumetric flask and the volume of the solution was brought to the mark with 96% ethanol (pinostrobin B reference solution). The optical density of test solution B of pinostrobin was measured on a spectrophotometer at 310 nm against the background of reference solution B of reference standard of pinostrobin.

Quantitative determination of total flavonoids in water-alcoholic extraction of licorice herb

About 1 ml of the obtained extraction was placed in a 50 ml volumetric flask, 2 ml of 3% alcohol solution of aluminum (III) chloride was added, the volume of solution was brought to the mark with 96% ethanol (test solution A), stirred and left for 40 min to form a flavonoid complex with aluminum. The solution obtained as follows was used as a reference solution: 1 ml of the extraction (1:50) was placed in a 50 ml measuring flask and the volume of the solution was brought to the mark with 96% ethanol (comparison solution A). Then the optical density of the test solution A was measured on a spectrophotometer at 310 nm against the background of the reference A.

The content of total flavonoids equivalent to pinocembrin and absolutely dry raw materials in percentage (X, %), is calculated by the formula:

$$x = \frac{D * m_0 * 50 * 50 * 1.05 * 100}{D_0 * m * 50 * 25 * (100 - W)},$$

where D – the optical density of the test solution; D_0 – the optical density of the pinostrobin SS; m – the mass of raw materials, g; m_0 – the mass loss in drying, %; 1.05 – the conversion factor.

In the absence of a pinostrobin standard sample, it is advisable to use the theoretical value of the specific absorption index, 680:

$$x = \frac{D * 50 * 25 * 100}{m * 680 * (100 - W)},$$

where D – the optical density of the test solution; m – the mass of raw materials, g; 680 – specific absorbance ($E_{1\,cm}^{1\%}$)of standard sample of pinocembrin at 310 nm; W – loss in weight during drying, %.

Validation of analytical methods

Validation of the developed methods was carries out according to the following indicators: specificity, linearity, precision (repeatability), intralaboratory precision, accuracy in accordance with the requirements of the Russian State Pharmacopoeia XIV edition. Microsoft Excel 2013 Software was used for calculations.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2023-11-2-127-136

RESULTS AND DISCUSSION

In the course of the experiment, water-alcoholic extracts of the licorice herb were obtained and their UV-spectra were studied. In the herb of this plant, flavanones prevail, the dominant flavonoid is pinocembrin (Fig. 1), which has a maximum absorption at a wavelength of ±2 nm (Fig. 2).

In our opinion, it is pinocembrin that mainly determines the character of the absorption curve of the water-alcohol extract from the licorice herb (Fig. 3). This conclusion is consistent with the literature data [7]. Since pinocembrin is not registered in the Russian Federation as a standard sample, the possibility of using pinostrobin (PhM 42-0073-01), close in chemical structure to pinocembrin as SS, was studied. It was determined that pinotembrine and pinostrobine have a maximum absorption at a wavelength of 290 nm (direct spectrophotometry) (Fig. 2 and 4). Taking into account the fact that in the case of the flavonoid sum content by direct spectrophotometry there is an overestimation of the experimental results, the possibility of using differential spectrophotometry with aluminum trichloride as a complexing reagent was studied.

It was found that the addition of aluminum (III) chloride to the test solution and the pinocembrin and pinostrobin solutions resulted in a bathochromic shift in the long-wave absorption spectrum (Fig. 2–4). At the same time, it was determined that differential spectrophotometry by the short-wave absorption maximum of pinostrobin and pinocembrin solutions are at wavelength 310 nm (Fig. 5 and 6), while in the long-wave region of the spectrum their absorption maxima do not coincide, which allows us to recommend 310 nm as an analytical wavelength. When using pinostrobin SS, the content of the flavonoid sum to pinocembrin was recalculated by introducing a coefficient into the formula for the calculation.

In the absence of pinostrobin SS, the theoretical value of pinostrobin specific absorption established experimentally, was used.

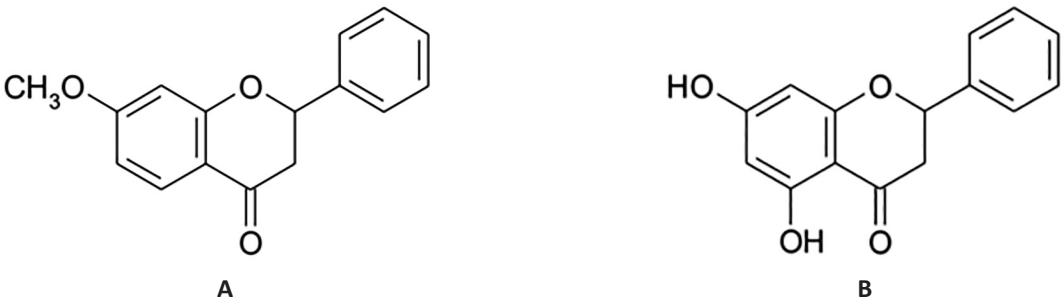
It was found that the total extraction of flavonoids from the licorice herb is achieved with 90% ethanol. The next step was to conduct an experiment to determine the optimal «raw material- extractant» ratio (1:50). Then the time parameters of the extraction, during which there was a maximum extraction of flavonoids from the raw materials were established − 60 min. The final step was to determine the degree of grinding of the raw material (2 mm), contributing to the full extraction of flavonoids by the extractant (Table 1).

The methods specificity was determined by the correspondence of the absorption maxima of the *Glycyrrhiza glabra* L. herb flavonoid complex and the solution of the standard pinostrobin sample with aluminum trichloride and the differential peak of the standard pinostrobin sample.

The methods linearity was determined for a series of pinostrobin solutions with concentrations ranging from 0.016 to 0.16 mg/ml (0.016; 0.032; 0.08; 0.16). Based on the data obtained, the dependence of the optical density values of pinostrobin solutions with aluminum trichloride on pinostrobin concentration was plotted, and then a linear regression equation was calculated (Fig. 8).

In studying the linear dependence of $y=bx+a$, the correlation coefficient was 0.9981, therefore, pinostrobin SS can be used to analyze the amount of flavonoids in the licorice herb in the indicated concentration range (Fig. 8).

The precision of the methods (a repeatability level) was estimated by analyzing the test sample of plant material in 11-fold repetition. The error of single determination of the amount of flavonoids in licorice herb with a 95% confidence level is ±0.73% (Table 2).



A B

Figure 1 – Structural formulas of pinostrobin (A) and pinocembrin (B)

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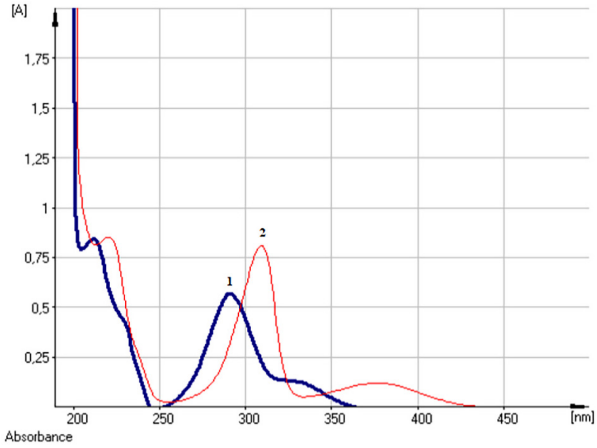

Figure 2 – Electronic spectra of solutions of water-alcohol solutions of pinocembrin
Note: 1 – pinocembrin solution (direct spectrophotometry),
2 – pinocembrin solution with addition of aluminum trichloride.

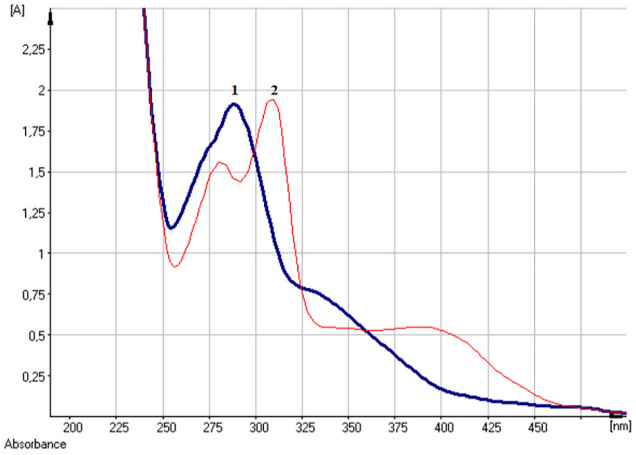

Figure 3 – Electronic spectra of water-alcohol extraction from *Glycyrrhiza glabra* L. herbs
Note: 1 – extraction solution (direct spectrophotometry),
2 – extraction solution with addition of aluminum trichloride.

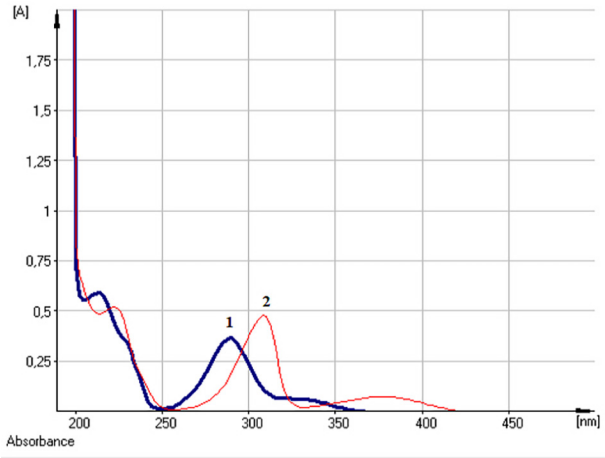

Figure 4 – Electronic spectra of pinostrobin solution
Note: 1 – pinostrobin solution (direct spectrophotometry),
2 – pinostrobin solution with addition of aluminum (III) chloride.

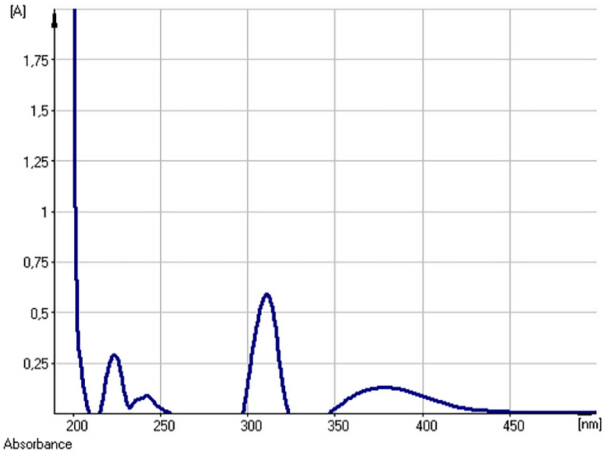

Figure 5 – Differential spectrum of pinocembrin solution

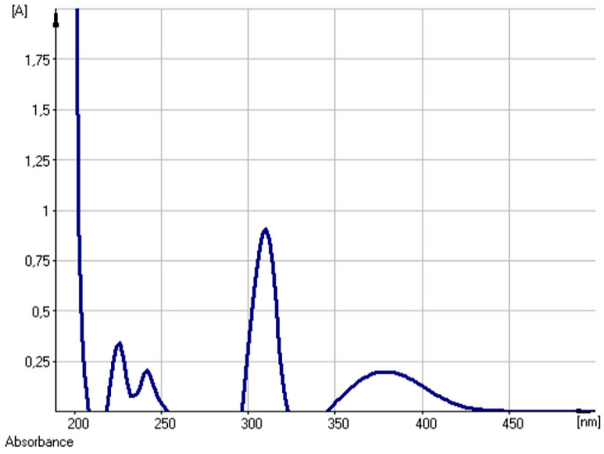

Figure 6 – Differential spectrum of pinostrobin solution

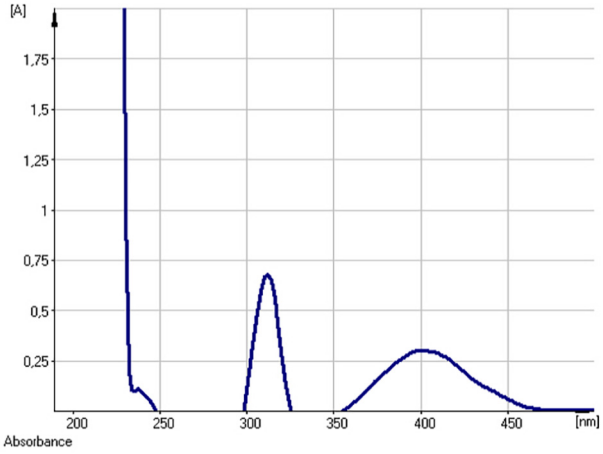

Figure 7 – Differential spectrum of water-alcohol extraction from *Glycyrrhiza glabra* L. herbs

ОРИГИНАЛЬНАЯ СТАТЬЯ
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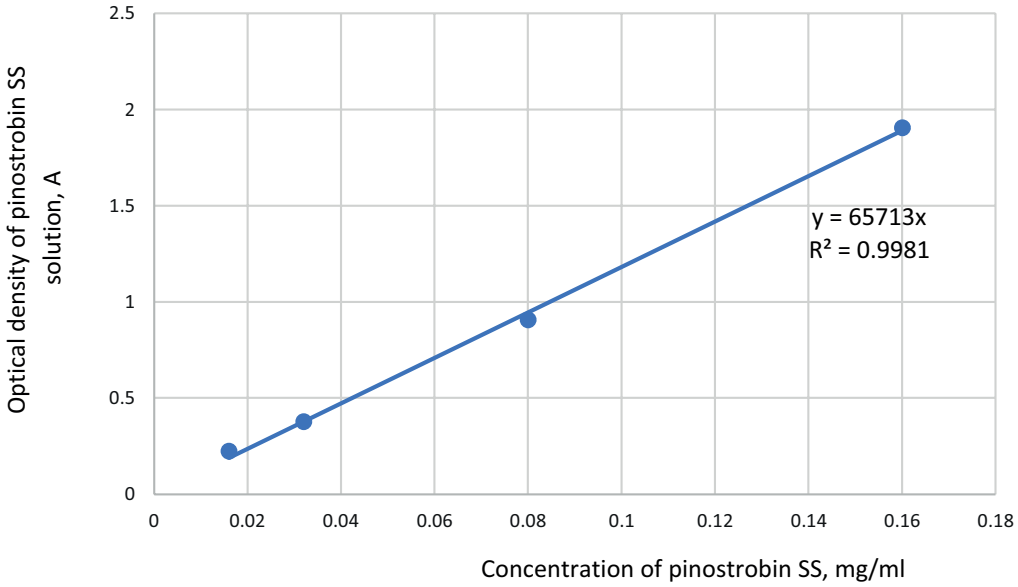

Figure 8 – Dependence of optical density values of pinostrobin solutions with aluminum (III) chloride on pinostrobin concentration (differential version)

Table 1 – Optimal extraction rates of total flavonoids from *Glycyrrhiza glabra* L. herbs at wavelength of 310 nm

Concentration of ethyl alcohol, %	«Raw materials-extractant» ratio	Extraction time, min	Degree of atomization, mm	Total flavonoids content equivalent to per pinocembrin and absolutely dry raw material, %
I. Extractor				
50	1:50	60	2	1.50±0.006
60				1.19±0.005
70				1.21±0.031
80				1.86±0.005
90				**2.28±0.001**
96				1.59±0.007
II. Extraction time				
90	1:50	30	2	2.02±0.008
		45		2.31±0.001
		60		**2.76±0.012**
		120		2.39±0.010
III. Degree of atomization				
90	1:50	60	1	3.10±0.013
			2	**3.41±0.015**
			3	2.52±0.011
IV. «Raw materials-extractant» ratio				
90	1:30	60	2	2.18±0.009
	1:50			**3.41±0.015**
	1:100			3.21±0.014

Table 2 – Precision estimation results of quantitative determination methods of total flavonoids in *Glycyrrhiza glabra* L. herbs (a repeatability level)

X, %	Metrological characteristics
3.40	
3.41	N=11
3.42	f=10
3.42	$\overline{X}$=3.41
3.45	SD=0.03690
3.46	RSD=1.0813%
3.39	$S_{\overline{X}}$=0.01113
3.47	P, %=95
3.35	t (P, t)=2.23
3.40	$\Delta\overline{X}$=0.02481%
3.37	E=0.73%

Table 3 – Validation of the laboratory precision of the methods for determining total flavonoids in *Glycyrrhiza glabra* L. herb

X, %	X, %	Metrological characteristics	
Analyst 1	Analyst 2	Analyst 1	Analyst 2
3.40	3.39	$\overline{X}$=3.43	$\overline{X}$=3.42
3.42	3.41	SD=0.02229	SD=0.02160
3.42	3.42	RSD=0.2652%	RSD=0.2579%
3.44	3.43	$S_{\overline{X}}$=0.009098	$S_{\overline{X}}$=0.008819
3.45	3.44	P, %=95	P, %=95
3.46	3.45	t (P, t)=2.23 (table)	t (P, t)=2.23 (table)
		E=0.59%	E=0.57%
		$\Delta\overline{X}$=0.02%	$\Delta\overline{X}$=0.019%

Notes: $t_{calculated}$=0.66<t (95%;6); $F_{calculated}$=1.06<F (95%;5;5) – differences between the results obtained are random.

Table 4 – Assessment results of the correctness of quantitative determination methods of total flavonoids in *Glycyrrhiza glabra* L. herbs

Injected pinostrobin, mg/ml	Found, mg/ml	Openness, %	Metrological characteristics
7.57	7.49	98.94	
7.57	7.52	99.34	$\overline{X}$=99.68%
7.57	7.56	99.87	SD=0.36062
15.15	15.08	99.54	RSD=0.3618%
15.15	15.11	99.74	$S_{\overline{X}}$=0.120206
15.15	15.16	100.07	P, % =95
22.73	22.66	99.69	t (P, t)=2.23
22.73	22.70	99.87	E=0.27%
22.73	22.74	100.04	$\Delta\overline{X}$=0.27%

Table 5 – Content of total flavonoids in *Glycyrrhiza glabra* L. samples of licorice (in %) equivalent to pinocembrin

No.	Characteristics of the raw material	Moisture content in raw plant materials, %	Content of total flavonoids in absolutely dry raw materials (%) equivalent pinocembrin
1.	Samara region (Kinelsky district, Alekseevka village), August 2021	9.97±0.003	3.38±0.015
2.	Samara city, Botanical Garden of Samara University, August 2021	9.96±0.003	0.48±0.002
3.	Orenburg region, (Sakmarsky district, Tatarskaya Kargala village), July 2017	9.99±0.003	0.39±0.002
4.	Republic of Kazakhstan, Derzhavinsk city, June 2018	10.01±0.002	1.34±0.006
5.	Samara region (Bolshechernigovsky district, Bolshaya Chernigovka village), August 2019	10.03±0.003	1.19±0.005

ОРИГИНАЛЬНАЯ СТАТЬЯ
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To assess in-laboratory precision, the analysis of the test sample was performed by another analyst on the same equipment on other days (Table 3). For each sample, the studies were carried out in six replications. Table 3 shows that the calculated value of Fisher's F-criterion 1.06 is less than the tabulated value of 5.05. Consequently, the variance of the analysis results of both chemistries are statistically equivalent and the differences between the obtained values not significant. Thus, the developed technique meets the validation requirements for the in-laboratory precision.

The correctness of the methods was determined by the standard addition methods. Pinostrobin solutions with the known concentration (25, 50 and 75%) were added to the aliquot of the test sample. The average opening percentage was 99.68±0.27% (Table 4). Three determinations were performed for each concentration. The error determined for the samples with additives of the SS was within the error of a single determination, indicating the absence of a systematic error (Table 4).

It was found that the average content of flavonoids in the studied sample of raw materials was 3.41±0.015% (the relative error of determination was ±0,73%).

Thus, based on the results of the validation evaluation of the experimental results, it can be concluded that this methods is suitable for the quantitative assessment of total flavonoids in terms of pinocembrin.

Using this methods, 5 samples of the licorice herb harvested in summer in different locations were analyzed (Table 5). It was determined that the content of total flavonoids in the analyzed samples varies from 0.39±0.002 to 3.41±0.015% with the moisture content of plant raw materials from 9.97±0.003 to 10.03±0.003% depending on the place of growth and the year of collection of plant raw materials (Table 5).

CONCLUSION

Thus, as a result of this study, the methods of quantitative determination of total flavonoids in the *Glycyrrhiza glabra* L. herb by differential spectrophotometry using a SS of pinostrobin at an analytical wavelength of 310 nm has been developed.

A validation assessment of the developed methods by the indicators of specificity, linearity, precision (a repeatability level), in-laboratory precision, correctness has been carried out. Based on the results of the validation assessment of the experimental results, these methods can be suitable for the quantitative assessment of total flavonoids equivalent to pinocembrin.

The results of the study can be used to create herbal medicines based on the licorice herb with a neuroprotective activity, antifibrotic effects, an antimicrobial action against *Esherichia coli* and a photoprotective efficacy.

The results of the study can be used in the development of a draft regulatory documentation for a promising type of raw materials «Licorice naked herb.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Olga A. Belova – collecting plant materials for analysis, experiment conducting, analyzing and interpreting the data obtained, preparing a draft manuscript, analyzing the literature, writing and preparing the manuscript for publication; Vladimir A. Kurkin – final approval of the manuscript for publication, results processing, verification of critical intellectual content; Maxim V. Egorov – participation in the development of the study concept and design, critical analysis of the study results.

All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

REFERENCES

1. Egorov MV, Kurkin VA, Zapesochnaya GG, Bykov VA. The qualitative and quantitative analysis of glycyrrhiza drugs and preparations. Bulletin of VSU. Series: Chemistry. Biology. Pharmacy. 2005;(1):175–80. Russian
2. Kurkin VA, Egorov MV. Standardization of *Glycyrrhiza glabra* L. roots and pharmaceutical "Glycyrrhizae liquid extract". Fundamental research. 2014;6(6):1232–6. Russian
3. Abdrakhmanova GM, Zhaugasheva SK, Pavelkovskaya GP. Technology development of new suppository on the basis of glycyrrhiza extract and paracetamol. Pharmacy and Pharmacology. 2014;7(6):36–8. DOI: 10.19163/2307-9266-2014-2-6(7)-36-38
4. Talanova IO, Volkova TG. A comparative analysis of some licorice-based expectorants. International Scientific Research Journal. 2023;129(3):1–5. DOI: 10.23670/IRJ.2023.129.29. Russian
5. Brovchenko BV, Ermakov VA, Bokov DE, Samylina IA, Demina NB, Chernova SV. Validation of the HPLC-UV Procedure For Determining The Content of Glycyrrhizic Acid In Licorice Roots. Khimiko-Farmatsevticheskii Zhurnal. 2019;53(12)42–7. DOI: 10.33380/2305-2066-2019-8-2-87-91. Russian
6. Brovchenko BV, Ermakova VA, Bokov DV, Samylina IA, Lazareva NB. Evaluation of Glycyrrhizic Acid Content in Licorice Roots and Products of its Processing by HPLC-UV

Method. Drug development & registration. 2019;8(2):87–91. DOI: 10.33380/2305-2066-2019-8-2-87-91. Russian
7. Ermakova VA, Samylina IA, Kovalev TY, Brovchenko BV, Dorovskikh EA, Bobkova NV. Licorice (Glycyrrhiza) roots: analysis of the requirements of the pharmacopoeia. Pharmacy. 2019;68(6):16–9. Russian
8. Yuldashev MP, Botirov EH, Vdovin AJ, Abdullaev AJ. Structural study of Glabrisoflavone, a Novel Isoflavone from *Glyccrrhiza glabra* L. Bioorganic Chemistry. 2000;26(11)873-6. Russian
9. Botirov EH, Kiyamitdinova F, Malikov VM. Flavonoids of aboveground part of *Glyccrhiza glabra.* Chemistry of natural compounds. 1986;(1):111–2. Russian
10. Gao M, Liu R, Zhu SY, Du GH. Acute neurovascular unit protective action of pinocembrin against permanent cerebral ischemia in rats. J Asian Nat Prod Res. 2008 May-Jun;10(5–6):551–8. DOI: 10.1080/10286020801966955
11. Liu R, Gao M, Yang ZH, Du GH. Pinocembrin protects rat brain against oxidation and apoptosis induced by ischemia-reperfusion both *in vivo* and *in vitro*. Brain Res. 2008 Jun 24;1216:104–15. DOI: 10.1016/j.brainres.2008.03.049
12. Rasul A, Millimouno FM, Ali Eltayb W, Ali M, Li J, Li X. Pinocembrin: a novel natural compound with versatile pharmacological and biological activities. Biomed Res Int. 2013;2013:379850. DOI: 10.1155/2013/379850
13. Shi LL, Qiang GF, Gao M, Zhang HA, Chen BN, Yu XY, Xuan ZH, Wang QY, Du GH. [Effect of pinocembrin on brain mitochondrial respiratory function]. Yao Xue Xue Bao. 2011 Jun;46(6):642–9. Chinese
14. Wu CX, Liu R, Gao M, Zhao G, Wu S, Wu CF, Du GH. Pinocembrin protects brain against ischemia/reperfusion injury by attenuating endoplasmic reticulum stress induced apoptosis. Neurosci Lett. 2013 Jun 24;546:57–62. DOI: 10.1016/j.neulet.2013.04.060
15. Li X, Zhai Y, Xi B, Ma W, Zhang J, Ma X, Miao Y, Zhao Y, Ning W, Zhou H, Yang C. Pinocembrin Ameliorates Skin Fibrosis via Inhibiting TGF-β1 Signaling Pathway. Biomolecules. 2021 Aug 19;11(8):1240. DOI: 10.3390/biom11081240
16. Said MM, Azab SS, Saeed NM, El-Demerdash E. Antifibrotic Mechanism of Pinocembrin: Impact on Oxidative Stress, Inflammation and TGF-β/Smad Inhibition in Rats. Ann Hepatol. 2018 Mar 1;17(2):307–17. DOI: 10.5604/01.3001.0010.8661
17. Promsan S, Jaikumkao K, Pongchaidecha A, Chattipakorn N, Chatsudthipong V, Arjinajarn P, Pompimon W, Lungkaphin A. Pinocembrin attenuates gentamicin-induced nephrotoxicity in rats. Can J Physiol Pharmacol. 2016 Aug;94(8):808–18. DOI: 10.1139/cjpp-2015-0468
18. Dunstan MS, Robinson CJ, Jervis AJ, Yan C, Carbonell P, Hollywood KA, Currin A, Swainston N, Feuvre RL, Micklefield J, Faulon JL, Breitling R, Turner N, Takano E, Scrutton NS. Engineering *Escherichia coli* towards *de novo* production of gatekeeper (2S)-flavanones: naringenin, pinocembrin, eriodictyol and homoeriodictyol. Synth Biol (Oxf). 2020 Aug 6;5(1):ysaa012. DOI: 10.1093/synbio/ysaa012
19. Cao W, Ma W, Wang X, Zhang B, Cao X, Chen K, Li Y, Ouyang P. Enhanced pinocembrin production in *Escherichia coli* by regulating cinnamic acid metabolism. Sci Rep. 2016 Sep 2;6:32640. DOI: 10.1038/srep32640
20. García Forero A, Villamizar Mantilla DA, Núñez LA, Ocazionez RE, Stashenko EE, Fuentes JL. Photoprotective and Antigenotoxic Effects of the Flavonoids Apigenin, Naringenin and Pinocembrin. Photochem Photobiol. 2019 Jul;95(4):1010–8. DOI: 10.1111/php.13085
21. Fuentes JL, Villamizar Mantilla DA, Flores González SJ, Núñez LA, Stashenko EE. Plants growing in Colombia as sources of active ingredients for sunscreens. Int J Radiat Biol. 2021;97(12):1705–15. DOI: 10.1080/09553002.2021.1987564
22. Gu X, Zhang Q, Du Q, Shen H, Zhu Z. Pinocembrin attenuates allergic airway inflammation via inhibition of NF-κB pathway in mice. Int Immunopharmacol. 2017 Dec;53:90–5. DOI: 10.1016/j.intimp.2017.10.005
23. Abdurahmanov BA, Sotimov GB, Khalilov RM, Mamatkhanov U. Technology for Obtaining a Substance Based on Flavonoids of The Aerial Part of *Glycyrrhiza glabra*. Khimiko-Farmatsevticheskii Zhurnal. 2021;55(10):18–22. DOI: 10.30906/0023-1134-2021-55-10-18-22. Russian
24. Khasanova RH, Nabiev AN, Vahabov AA. Pinocembrin preparation of licorice holodendron. Scientific and Practical Journal "Gastroenterology of St. Petersburg". 2003;(2–3):180. Russian
25. Mamatkhanova MA, Abdurakhmanov BA, Nigmatullaev BA, Sotimov GB, Khalilov RM, Mamatkhanov U. Study of aerial parts of *Glycyrrhiza glabra* as promising raw materials for the production of drugs. Chemistry of plant raw material. 2016;(1):171–6. Russian

AUTHORS

Olga A. Belova – postgraduate student of the Department of Pharmacognosy with Botany and Basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-4767-0824. E-mail: belova_oa@pranapharm.ru

Vladimir A. Kurkin – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Pharmacognosy with Botany and Basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-7513-9352. E-mail: v.a.kurkin@samsmu.ru

Maxim V. Egorov – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Pharmacognosy with Botany and Basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0001-9441-2628. E-mail: m.v.egorov@samsmu.ru