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Development and validation of method of quantitative determination of flavonoids from the above-ground part of *Glycyrrhiza glabra* L.

The aim of this study was to determine the optimal extraction factors of flavonoids from the above-ground part of licorice (Glycyrrhiza glabra L.) and to develop a method for their quantitative determination by differential spectrophotometry. The object of the study was collected in the Volgograd region during flowering and dried samples of the herb of the medicinal plant. Ethyl alcohol 70% was chosen as the optimal extractant of flavonoids from the studied raw materials. It was found that the extraction of the maximum amount of flavonoid fraction is observed at three times extraction of plant raw material for 30 minutes each in the ratio with extractant 1:30. Quantitative determination of flavonoid compounds was performed by measuring the optical density of coloured complexes formed in extracts from the above-ground part of G. glabra L. with 2.5% aluminum chloride solution. Detection of the optical density of the tested solutions was conducted at a wavelength of 408 nm, corresponding to the maximum of the standard sample solution of rutin (Sigma, USA) after the addition of 2.5% aluminum chloride solution. Determined by differential spectrophotometry method the content of the sum of flavonoids in recalculation on rutin in extracts from the studied samples of plant raw materials of G. glabra L., obtained under the selected conditions, is 4.34±0.06%. The relative error of determination did not exceed 5%. A validation analysis was also conducted, the results of which showed the specificity, linearity and precision of the developed methodology. The studied validation parameters were within the acceptance criteria.

Keywords: Glycyrrhiza glabra L., licorice, plant raw material, above-ground part, rutin, differential spectrophotometry.

Introduction

The above-ground part of licorice (*Glycyrrhiza glabra* L.) of the *Fabaceae* family is a potential object of pharmacognostic study for obtaining highly effective therapeutic and preventive drugs [1, 2]. It is known that the green parts of the medicinal plant contain di- and polysaccharides, triterpene saponins, tannins, vitamins, amino acids, flavonoids (pinocembrin, rutin, quercetin, vitexin, etc.) [3–5]. However, for the successful introduction of herb of *G. glabra* into practical medicine and optimization of the use of this morphological group of plant raw materials in the therapy of various diseases, it is necessary to develop complex approaches to its standardization.

Due to the fact that the formation of the main pharmacological properties (antimicrobial, anti-inflammatory [6, 7], etc.) of this plant raw material is directly related to the presence of flavonoids in the herb of *G. glabra*, it is rational to study the dynamics of accumulation of compounds of this group, as well as the development of approaches and techniques for their quantitative determination [8, 9]. At the same time, an important stage is the selection of optimal extraction conditions of the basic group of biologically active substances, allowing to achieve maximum yield from plant objects.

Therefore, the aim of this study was to determine the influence of some factors (type of extractant, time and extraction multiplicity) on the process of flavonoids extraction from the above-ground part of licorice and their practical application in the development of quantitative determination methods in the plant raw material.

Experimental

Samples of the above-ground part (air-dried raw material) of *G. glabra*, collected in the Volgograd region from wild populations of the medicinal plant in late June – early July 2023 during the period of flowering of the medicinal plant, were used in this study.

The yield of flavonoid fraction from plant raw materials is influenced by the type of extractant, time and multiplicity of extraction. Traditionally, extraction of flavonoid fraction in the development of quantitative determination methodology in plant raw materials is conducted with ethyl alcohol. In this study, ethyl

alcohol of different concentrations (30%, 50%, 70%, and 95%) was used as an extractant. The optimal concentration was selected at which the highest yield of flavonoids was observed.

1.0 g of licorice herb ground to 1 mm was placed in a flat-bottomed flask, 30 ml of ethyl alcohol of appropriate concentration was added. The flask with the contents was weighed, connected to a condenser and the raw material was extracted for 30 minutes. Then the flask was cooled, weighed and the mass was brought to the original weight with ethyl alcohol. The obtained extract was filtered (solution A) and used for quantitative determination of flavonoid amount by differential spectrophotometry. 2.5 ml of solution A was placed in a 25 ml volumetric flask, 2.5 ml of 2.5% aluminum chloride and 0.1 ml of diluted acetic acid were added. In another flask 2.5 mL of solution A and 01, mL of dilute acetic acid were placed. Both flasks were brought to the mark with alcohol of appropriate concentration (solution B) and measured the optical density of the studied solutions spectrophotometer Shimadzu UV-1800 in quartz cuvettes with a layer thickness of 10 mm at the analytical wavelength corresponding to the wavelength of the standard sample solution of rutin used in the work (408±2 nm).

In parallel, the optical density of rutin standard sample solution (Sigma, USA) was measured according to the same principle. For the preparation of solution A about 0.05 g (exact weight) of rutin was placed in a measuring flask with a capacity of 100 ml and brought with ethyl alcohol 95% to the mark.

The optical density of the test extract with aluminum chloride was measured relative to the control (extract without addition of aluminum chloride solution).

Calculation of the quantitative content of flavonoids (X, %) in recalculation on rutin in absolutely dry raw material was performed according to the formula:

$$X = \frac{A \cdot V_1 \cdot 25 \cdot 100}{A_{1\text{см}}^{1\%} \cdot m \cdot V_2 \cdot (100 - W)},$$

where A – optical density of the tested solution;
$A_{1\text{см}}^{1\%}$ – specific absorption index of rutin solution;
V_1 – volume of extractant;
V_2 – volume of aliquot for preparation of solution B;
m – weight of raw material, g;
W – moisture content of plant raw material, %.

Then the optimal extraction time and extraction multiplicity were selected.

Statistical processing of experimental data was performed using Microsoft Office Excel computer program. Each test was done three times (P = 95%; n = 3).

Validation of the method for quantitative determination of flavonoids in the above-ground part of *G. glabra* was performed according to the following indicators: specificity, linearity, precision (repeatability).

Specificity of the method was determined by the position of maxima of light absorption of flavonoids complexation reaction products of alcoholic extracts from licorice and standard sample of rutin with aluminum chloride on differential spectra.

Determination of the linearity of the method included the study of the dependence of the values of optical density of the stained complexes of flavonoids of the studied extracts from the above-ground part of the medicinal plant with aluminum chloride on their concentration. For this purpose, five dilutions of alcoholic extract were prepared. In five volumetric flasks (25 ml) were placed 1.0 ml; 1.5 ml; 2.0 ml; 2.5 and 3.0 ml of the extract, 10 ml of 95% ethyl alcohol, 2.5 ml of 2.5% alcoholic solution of aluminum chloride were added. The optical density of the obtained solutions was measured and the dependence diagram was plotted.

The precision (repeatability) of the method was determined by taking six exact weights of herb of *G. glabra*, preparing six alcoholic extracts according to the developed method and determining the content of the sum of flavonoids in the tested samples of raw materials.

Results and Discussion

The dominance of rutin in the above-ground part of *G. glabra* was previously established by HPLC [10]. Due to the fact, this flavonoid has a significant role in the formation of the spectral characteristics of the tested extracts from the herb of this medicinal plant. When studying their differential spectra, it was found that the stable absorption maximum is observed at a wavelength of 407±2 nm and is similar to the maximum of the solution of the standard sample of rutin used in the work (408±2 nm) (Fig. 1).

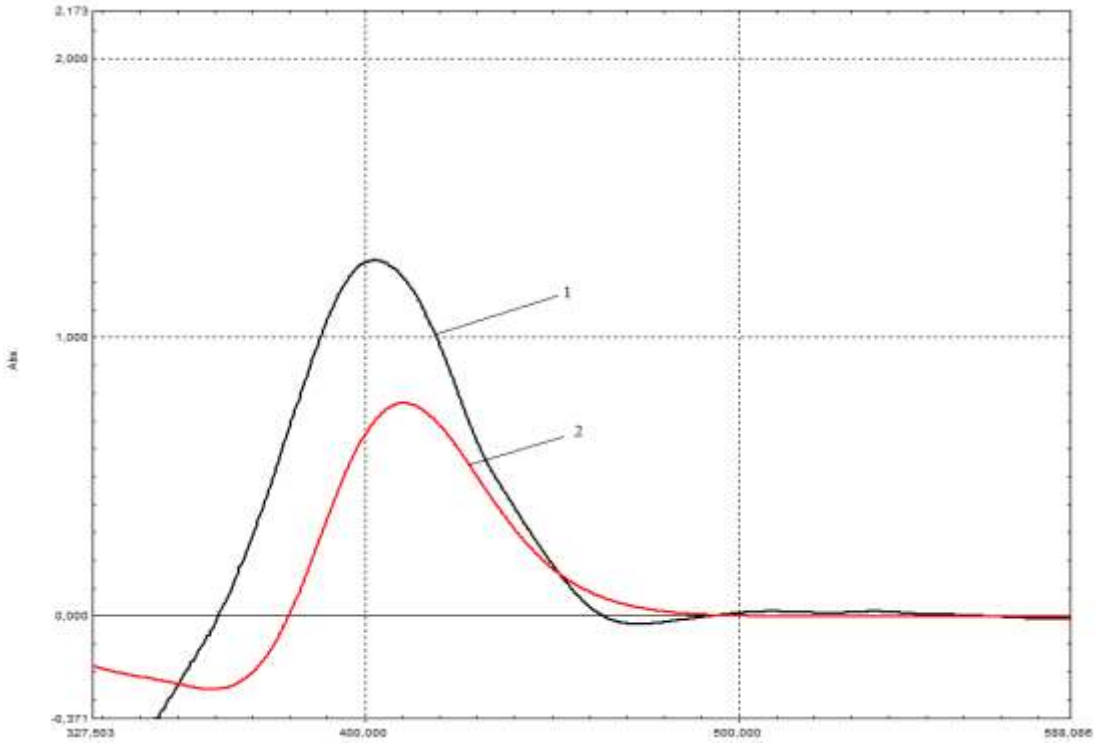

Figure 1. Differential spectra of alcohol extracts from the herb of *Glycyrrhiza glabra* (1) and rutin standard sample solution (2)

This region of the spectrum is quite different from the absorption spectra of other groups of phenolic compounds, which are present in extracts from plant raw materials, and improves the selectivity of the determination. This fact is taken as a basis for the developed method. Quantitative determination of the sum of flavonoids in the above-ground part of *G. glabra* was conducted in recalculation on rutin. The wavelength at which the maximum of the standard sample was observed was chosen as the analytical wavelength.

Data on the influence of extractant concentration on the yield of this group of biologically active substances from herb of *G. glabra* are presented in Table 1.

Table 1

The effect of ethyl alcohol concentration on the yield of flavonoids from the herb of *Glycyrrhiza glabra* L.

Ethyl alcohol concentration	Flavonoids content, %
30%	2,68±0,08
50%	3,01±0,04
70%	3,30±0,09
95%	3,18±0,04

It was found that the maximum value of the amount of flavonoids in recalculation on rutin in the extracts is observed when extracting plant material *G. glabra* with ethyl alcohol 70%. Therefore, further studies were performed using ethyl alcohol of this concentration.

Tables 2 and 3 show the results of the effect of the multiplicity and time of extraction with ethyl alcohol 70% on the yield of flavonoid fraction from the tested samples of plant raw materials.

Table 2

Effect of extraction time with 70% ethyl alcohol on the yield of flavonoids from the herb of *Glycyrrhiza glabra* L.

Extraction time	Flavonoids content, %
30 min	3,27±0,04
60 min	3,65±0,09
90 min	3,98±0,11

Table 3

Effect of extraction multiplicity on the yield of flavonoids from the herb of *Glycyrrhiza glabra* L.

Extraction multiplicity	Flavonoids content, %
1	4,04±0,09
1/2	4,24±0,07
1/3	4,33±0,09

The obtained results indicate that the optimal factors of extraction are three times extraction of the exact weight (1.0) of the plant raw material (herb of *G. glabra*) crushed to 1 mm for 30 minutes with ethyl alcohol 70%. The content of the sum of flavonoids in recalculation on rutin in extracts from the above-ground part of the medicinal plant obtained under these conditions, determined by differential spectrophotometry, was 4.34±0.06% (with a confidence level of 95%). The metrological characteristic of the experiment is presented in Table 4.

Table 4

Metrological characterization of the quantitative determination of the content of the sum of flavonoids in recalculation on rutin in the herb of *Glycyrrhiza glabra* L.

f	Xcp	S	P, %	t (P, f)	ΔX	E, %
5	4,34	0,05	95	2,57	0,06	3,38

Linear regression parameters were calculated in Microsoft Office Excel programme. The dependence of the optical density value of the tested extracts on the content of the sum of flavonoids in recalculation on rutin (%) is described by the equation y=0,505x+2,193 (Fig. 2). R^2 is 0.996, which confirms the linear dependence in the studied range of concentrations.

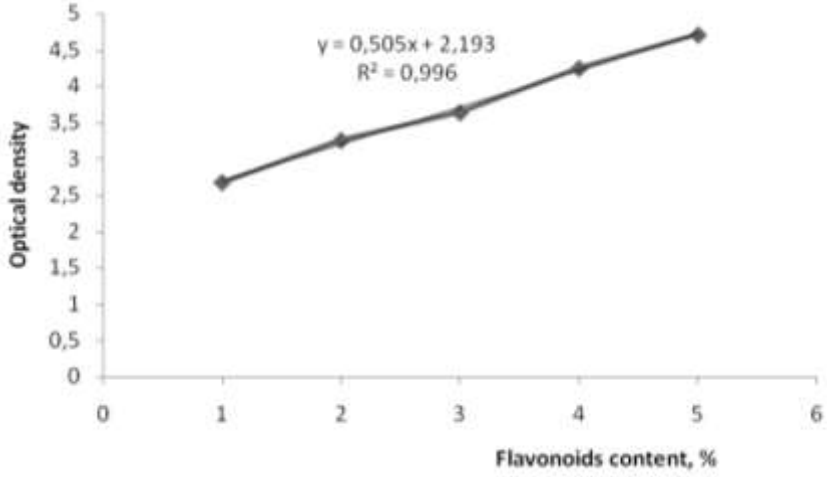

Figure 2. Dependence of optical density of extracts from the above-ground part of *Glycyrrhiza glabra* on the content of the sum of flavonoids in recalculation on rutin

Determination of precision (reproducibility) indicates the repeatability of this method. The relative error (E, %) was 3.38%.

Conclusions

Thus, in the course of the study, the optimal parameters affecting the extraction of flavonoids from herb of *G. glabra* L. were determined. The obtained data were used in the development of methods for quantitative determination of compounds of this group of biologically active substances in the examined plant raw materials. It was found that the maximum yield of the sum of flavonoids in recalculation on rutin is observed at three times extraction on 30 minutes of samples of licorice herb 70% ethyl alcohol and on the average was 4,34±0,06%.

Validation evaluation of the developed method was also carried out in terms of specificity, linearity, precision (repeatability), the results of which are within the criteria of acceptability.

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О.В. Недилко, А.В. Яницкая

Glycyrrhiza glabra L. жерүсті мүшелеріндегі флавоноидтарды сандық анықтау әдістерін әзірлеу және валидациялау

Зерттеудің мақсаты — мия тамырының (*Glycyrrhiza glabra* L.) жерүсті бөлігінен флавоноидтарды алудың оңтайлы коэффициенттерін анықтау және оларды дифференциалды спектрофотометрия әдісімен сандық анықтау әдістемесін әзірлеу. Зерттеу нысаны ретінде Волгоград облысында гүлдену кезінде жиналған және дәрілік өсімдік шөптерінің кептірілген үлгілері алынған. Зерттелетін шикізаттан флавоноидтардың оңтайлы экстрагенті ретінде 70% этил спирті таңдалды. Флавоноидты фракцияның максималды мөлшерін алу өсімдік шикізатын экстрагент 1:30 қатынасында 30 минуттан үш рет алу кезінде байқалатыны анықталды. Флавоноидты қосылыстарды сандық анықтау *G. glabra* L. жерүсті бөлігінен алынған сығындыларда түзілген боялған кешендердің оптикалық тығыздығын өлшеу 2,5 % алюминий хлоридінің ерітіндісі арқылы жүргізілді. Зерттелетін ерітінділердің оптикалық тығыздығын анықтау 2,5% алюминий хлоридінің ерітіндісін қосқаннан кейін стандартты рутин үлгісі (Sigma, АҚШ) ерітіндісінің максимумына сәйкес келетін 408 нм толқын ұзындығында жүргізілді. Дифференциалды спектрофотометрия әдісімен анықталған, таңдалған жағдайларда алынған G. glabra L. өсімдік шикізатының зерттелетін үлгілерінен алынған сығындылардағы флавоноидтардың рутиндік мөлшері 4,34±0,06% құрайды. Анықтаудың салыстырмалы қателігі 5%-дан аспады**.** Сондай-ақ, валидациялық талдау жүргізілді, оның нәтижелері әзірленген әдістеменің ерекшелігін, сызықтығын және дәлдігін көрсетті. Тексерудің зерттелген параметрлері жарамдылық критерийлері шегінде болды.

Кілт сөздер: Glycyrrhiza glabra L., мия тамыры, өсімдік шикізаты, жерүсті мүшелері, рутин, дифференциалды спектрофотометрия.

О.В. Недилко, А.В. Яницкая

Разработка и валидация метода количественного определения флавоноидов в надземных органах *Glycyrrhiza glabra* L.

Цель исследования — определение оптимальных коэффициентов извлечения флавоноидов из надземной части солодки (*Glycyrrhiza glabra* L.) и разработка методики их количественного определения методом дифференциальной спектрофотометрии. Объектом исследования служили собранные в Волгоградской области во время цветения и высушенные образцы травы лекарственного растения. В качестве оптимального экстрагента флавоноидов из исследуемого сырья был выбран спирт этиловый 70

%. Установлено, что извлечение максимального количества флавоноидной фракции наблюдается при трехкратном экстрагировании растительного сырья по 30 мин в соотношении с экстрагентом 1:30. Количественное определение флавоноидных соединений проводили путем измерения оптической плотности окрашенных комплексов, образующихся в экстрактах из надземной части *G. glabra* L., с 2,5 % раствором алюминия хлорида. Определение оптической плотности исследуемых растворов проводили при длине волны 408 нм, соответствующей максимуму раствора стандартного образца рутина (Sigma, США) после добавления 2,5 % раствора хлорида алюминия. Определенное методом дифференциальной спектрофотометрии содержание суммы флавоноидов в пересчете на рутин в экстрактах из исследуемых образцов растительного сырья *G. glabra* L., полученных в выбранных условиях, составляет 4,34±0,06 %. Относительная ошибка определения не превышала 5 %. Также был проведен валидационный анализ, результаты которого показали специфичность, линейность и прецизионность разработанной методики. Исследованные параметры валидации находились в пределах критериев приемлемости.

Ключевые слова: Glycyrrhiza glabra L., солодковый корень, растительное сырье, надземные органы, рутин, дифференциальная спектрофотометрия.

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