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Х А Б А Р Л А Р Ы

ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН
АО «Институт топлива, катализа и
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NAS RK is pleased to announce that News of NAS RK. Series of chemistry and technologies scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of News of NAS RK. Series of chemistry and technologies in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential content of chemical sciences to our community.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабарлары. Химия және технология сериясы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Web of Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Химия және технология сериясы Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді химиялық ғылымдар бойынша контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Известия НАН РК. Серия химии и технологий» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Известия НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному контенту по химическим наукам для нашего сообщества.

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OPTIMIZATION OF METHODS OF QUANTITATIVE DETERMINING FLAVANOIDS IN KNOTWEED RAW MATERIAL

Abstract. The method of quantitative determination of flavonoids in the grass of the mountaineer avian is developed. Improvement of the existing Pharmacopoeia technique for determining the total content of flavonoids in the grass of the mountain bird is an urgent task, since the long procedure of extraction of raw materials of the bird mountain and the maximum extract of flavonoids. The optimal conditions of the experiment were chosen. Parameter optimization of pharmacopoeial methods is the reduction of time of the extraction of flavonoids (total extraction time 1.5 hours) with the introduction of the calculation formula of the correction factor for the incomplete extraction. The content of flavonoids in the grass of the bird Highlander determined by Pharmacopoeia was $1.62 \pm 0.02\%$. The content of the amount of flavonoids is normalized in the raw material at a level of not less than 0.5%. To determine the metrological characteristics of the developed technique, 10 parallel definitions were carried out. The relative error of definition does not exceed 3,13 %. A rapid method for determination of flavonoids in poultry sold in buckets for 5 minutes in a variant of the extraction with 70% ethanol. The definition error does not exceed 3.13 %.

Keywords: flavonoids, quantification, UV-spectrophotometry, Herba Polygonum aviculare.

Introduction

Polygonum aviculare L (knotweed) possesses a wide range of biological activity. The drug from knotweed is used to stop bleeding, inflammation of the urinary tract and stone binding in the kidney. Knotweed is an annual herb of the buckwheat family. The root is rod-like, low- branched. Stems are 10-60 cm long, spread on the soil or ascending, often branched from the base. The leaves are from elliptical to linear-lanceolate form, narrowed at the base in a short petiole, 1-4 cm long and 0.5-2 cm wide. The flowers are located in the leaf axils 2-5. The perianth is deeply dissected, five-membered, green at the bottom, white or pink at the top. The fruit is a triangular, black, occasionally chestnut nutlet. It flows and fruits from May to late autumn, in Western Siberia from mid-June to September.

Flavonoids are a large class of natural compounds, the variety of which is mainly caused by the structure of the aglycone (oxidation state of the three-carbon fragment, the position of the side phenyl radical, the size of the heterocycle and other features), as well as the composition of the glycoside fragment.

Physical properties. Flavonoids are crystalline substances with a specific melting point, odorless, having a yellow color (flavones, flavonols), colorless (isoflavones, flavanones).

The group of flavonoids also includes anthocyanins (natural dyes of plants), which are colored differently depending on the pH of the medium: in an acidic medium, they are red (salts of cations), in alkaline blue (salts of anions) [1].

The grass of knotweed is included in the USSR State Pharmacopoeia XI edition and the European Pharmacopoeia, the Pharmacopoeia of Kazakhstan. All the regulatory documents provide for standardization of raw materials by the content of flavonoids. However, there is a difference in the methods of quantifying these compounds in the given raw material. The domestic pharmacopoeia provides for triple extraction of knotweed flavonoids (in the form of glycosides) with 70% alcohol (each extraction

lasting 30 minutes), followed by adding aluminum chloride solution to the aliquot of the extract and measuring the optical density of the solution at 410 nm after 20 minutes. For the calculations, the specific absorption index of the avicularin complex with aluminum chloride at 410 nm is equal to 330. The total content of flavonoids is normalized in raw materials at the level of at least 0.5%. The European Pharmacopoeia uses a unified method. The essence of the method lies in the fact that a sample of the raw material is subjected to acid hydrolysis in acetone, the obtained aglycones are extracted with ethyl acetate and the optical density of the complex of aglycones with aluminum chloride in ethyl acetate – methanol – acetic acid is measured. The content of flavonoids is calculated using the specific absorption rate of hyperoside that is 500 under the conditions of determining. The content of flavonoids is normalized in raw materials at the level of at least 0.3% [2].

The opportunities for improving the existing pharmacopoeial methodology of determining the total content of flavonoids in the knotweed grass lie in the area of optimizing a rather long procedure of flavonoid extraction (1.5 hours), when you can abandon the exhaustive extraction mode of the detected substances and enter a correction factor for incomplete extraction into the calculation formula [3].

Experimental part

The parameter of optimization of the pharmacopoeial procedure is reducing the time of extraction of flavonoids (the total extraction time is 1.5 hours) with introducing a correction factor for the incompleteness of extraction into the calculation formula. The content of flavonoids in the knotweed grass determined by the pharmacopoeial method was $1.62 \pm 0.02\%$.

The main parameter of optimization of the pharmacopoeial method for determining the content of flavonoids in the knotweed grass is reducing the extraction time, alongside with such parameters as the concentration of alcohol used for the extraction of flavonoids and the maximum absorption of the complex of flavonoids with aluminum chloride was tested under the experimental conditions. The dependence of the yield of flavonoids on the concentration of alcohol and the time of extraction was tested in experiments with at least 4 repetitions. Since 70% ethyl alcohol is used in the pharmacopoeial procedure for extraction, this concentration and extreme concentrations used for the extraction of flavonoids, i.e. 40% and 95%, were tested. The results are presented in Table 1 [4-5].

Table 1 -Dynamics of extracting flavonoids from the knotweed grass depending on the extraction time at different concentrations of alcohol (with the ratio of raw materials/extractant equal to 1:100), %

The yield of flavonoids, (% by weight of dry raw materials)/(% of the total amount in raw materials)	Alcohol concentration, (%)	Extraction time, min.			
		5	10	15	30
	40	1.32±0.03 81	1.33±0.03 82	1.37±0.02 85	1.50±0.05 93
	70	1.51±0.04 93	1.52±0.03 94	1.53±0.03 94	1.55±0.03 96
	95	0.37±0.02 23	0.46±0.02 28	0.56±0.02 35	0.79±0.04 49

The results obtained indicate that 70% of the ethanol concentration is optimal in the studied range. In this case, the bulk of flavonoids are removed by the solvent already within the first minutes of extraction, and subsequently, few residues are extracted [6].

To check the maximum absorption of the products of the knotweed flavonoids reaction with aluminum chloride, we recorded the electronic spectra of the extraction itself, the reaction products of flavonoids with aluminum chloride against the background of 95% alcohol and against the extraction itself (differential spectrum). The long-wavelength maximum of the differential spectrum was at the mark of 407 nm, which fits into the permissible deviation intervals (± 5 nm), so all measurements were carried out at the wavelength specified in the pharmacopoeial method, 410 nm. All of the above makes it possible to offer an express method for determining flavonoids in the knotweed grass, when the bulk of these compounds are extracted from raw materials within a relatively short period of time and instead of a long exhaustive extraction of flavonoids remaining in the pile, a correction factor for extraction incompleteness is entered into the calculation formula [7].

Results and discussion

Express-method of quantitative determining flavonoids in the knotweed grass. The analytical sample of the raw material is crushed to the size of particles passing through the sieve with holes 1 mm in

diameter. About 1 g (exact weight) of the crushed raw material is placed in a 250 ml thin flask, 100 ml of 70% alcohol are added, the flask is attached to the reflux condenser and heated in a boiling water bath within 5 minutes after boiling the solvent. Then the flask is cooled to the room temperature under a stream of cold water and filtered through a filter paper into a measuring cylinder with the capacity of 100 ml (solution A). 4 ml of the A solution are placed in a 25 ml volumetric flask, 2 ml of a 2% solution of aluminum chloride in 95% alcohol are added and the volume of the solution is brought to the mark with 95% alcohol; after 20 min. the optical density of the solution is measured on the spectrophotometer at the wavelength of 410 nm in a cell with a layer thickness of 10 mm. The following solution is used as the reference solution: 4 ml of the A solution are placed in a measuring flask with the capacity of 25 ml, 1 drop of diluted hydrochloric acid is added and the volume of the solution is adjusted to the mark with 95% alcohol. The content of the total flavonoids in terms of avicularin and absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{D * V * 25 * 100 * 1.07}{330 * m * 4 * (100 - W)}$$

where D is the optical density of the test solution; 330 is the specific absorption coefficient of the complex of avicularin with aluminum chloride at 410 nm; V is the volume of the A solution in the measuring cylinder; m is the mass of raw material in grams; 1.07 is the correction factor for incomplete extraction of flavonoids; W is the mass loss during the raw materials drying in percent.

To determine metrological characteristics of the developed methodology, 10 parallel determinations were carried out (Table 2). The method error does not exceed 3.13%.

Table 2 –Metrological characteristics of the express-method of determining flavonoids in the knotweed grass

f	X	S ²	S	P	t	(0.95; 9)	Δx е. %
9	1.62	0.005055	0.0711	0.95	2.26	0.05	3.13

Thus, the express-method of determining flavonoids in the knotweed grass is implemented in the variant of extraction with 70% ethyl alcohol within 5 minutes. The determination error does not exceed 3.13% [8].

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ҚЫЗЫЛ ТАСПА ШИКІЗАТЫНДА ФЛОВАНОИДТАРДЫ САНДЫҚ АНЫҚТАУ ӘДІСТЕМЕСІН ОҢТАЙЛАНДЫРУ

Аннотация. Қызыл таспа шөмішіндегі флавоноидтардың сандық анықтау әдістемесі әзірленді. Қызыл таспа шөмішіндегі қосынды құрамындағы флавоноидтерді анықтаудың қазіргі фармакопоялық әдістемесін жетілдіру өзекті міндет болып табылады, өйткені қызыл таспа шикізаты ұзақ процедуралы экстракция және флавоноидтер максимум түрде алынады. Эксперимент жүргізудің оңтайлы шарттары алынды. Фармакопоялық Әдістеменің оңтайландыру параметрі флавоноидтарды экстракциялау уақытын (экстракцияның жалпы уақыты 1,5 сағат) есептеу формуласына экстракцияның толық еместігіне түзету коэффициентін енгізумен қысқарту болып табылады. Фармакопоялық әдіспен анықталған құс қыша шөмішіндегі флавоноидтардың мөлшері $1,62 \pm 0,02\%$ құрады. Флавоноидтар сомағының құрамы шикізатта кемінде 0,5% деңгейінде нормаланады. Әзірленген Әдістеменің метрологиялық сипаттамаларын анықтау үшін 10 параллельді анықтау жүргізілді. Анықтаудың салыстырмалы қатесі 3,13 % аспайды. Қызыл таспа шөмішіндегі флавоноидтарды анықтаудың Экспресс-әдісі 70% этил спиртімен экстракция нұсқасында 5 минутта іске асырылады. Анықтау қатесі 3,13 % аспайды.

Түйін сөздер: флавоноидтар, сандық анықтау, УК-спектрофотометрия, қызыл таспашөбі.

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ОПТИМИЗАЦИЯ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ФЛОВАНОИДОВ В СЫРЬЕ ГОРЦА ПТИЧЬЕГО

Аннотация. Разработана методика количественного определения флавоноидов в траве горца птичьего. Усовершенствование существующей фармакопейной методики определения суммарного содержания флавоноидов

в траве горца птичьего является актуальной задачей так как продолжительная процедура экстракции сырья птичьего горца и максимум извлечь флавоноиды. Подобраны оптимальные условия проведения эксперимента. Параметром оптимизации фармакопейной методики является сокращение времени экстракции флавоноидов (общее время экстракции 1,5 часа) с введением в расчетную формулу поправочного коэффициента на неполноту экстракции. Содержание флавоноидов в траве горца птичьего, определенное фармакопейным методом, составило $1,62 \pm 0,02\%$. Содержание суммы флавоноидов нормируется в сырье на уровне не менее 0,5%. Для определения метрологических характеристик разработанной методики провели 10 параллельных определений. Относительная ошибка определения не превышает 3,13 %. Экспресс-метод определения флавоноидов в траве горца птичьего реализуется в варианте экстракции 70% этиловым спиртом в течение 5 минут. Ошибка определения не превышает 3,13 %.

Ключевые слова: флавоноиды, количественное определение, УФ – спектрофотометрия, трава горца птичьего.

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