Development and validation of a method for the quantitative determination of the amount of flavonoids in grass shepherd's purse

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Development and validation the method's of quanti-tative determination of flavonoids' sum in Capsella bursa - pastoris (l.) Medik. Materials

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RESUME

Nowaydays there isn't any method for quantitative definition of biologically active substances in the normative documentation for Capsella bursa - pastoris (L.) Medik. materials. As the requirements for the crude drugs' standardization increase steadily it is necessary to make a quantitative assessment of biologically active substances. This study's aim was to work out a method for Capsella bursa – pastoris (L.) Medik. materials crude quantitative assessment and its subsequent validation. The research team devised a method for Capsella bursa – pastoris materials active substances' quantitative determination. Application criteria for the accuracy, repeatability, reproducibility and linearity method were also determined. This method for Capsella bursa – pastoris materials active substances' quantitative determination was to the validation of compendial method. A norm of biologically active substances' content in nettle leaves was proposed. The results of the study were included in the normative documentation for “Krasnogorskleksredstva” House monography.

Keywords: Capsella bursa – pastoris, flavonoids, quantitative determination.

SUMMARY

Currently, there is no method for the quantitative determination of biologically active substances in the ND for the herb of shepherd's purse. The article presents studies on the development of a method for the quantitative determination of the amount of flavonoids in the herb of shepherd's purse by the method of differential spectrophotometry. The proposed method was also validated. The criteria for the acceptability of the developed method are established. The norm for the content of biologically active substances in the raw material of the shepherd's purse has been proposed. The results obtained are included in the FSP of Krasnogorskleksredstva OJSC, Shepherd's purse grass.

Key words: shepherd's purse, flavonoids, quantitative determination.

Introduction

At present, according to the normative document on the herb of shepherd's purse, the quality of raw materials is determined by the content of extractives extracted by 70% ethanol. The ever-growing requirements for the standardization of medicinal plant materials necessitate a quantitative assessment of the content of biologically active substances.

Numerous literature data indicate the presence of flavonoid compounds in the raw material of the shepherd's purse — these are glycosides of quercetin, luteolin, diosmetin [1]; as well as rutin, luteolin, diosmin, quercetin, hesperidin, luteolin 7-rutinoside, luteolin 7-glucogalactoside [2, 3].

The content of flavonoids is largely responsible for the pharmacological activity of the herb preparations of shepherd's purse. Thus, it has been shown that the amount of flavonoids in the herb of shepherd's purse affects the permeability of the walls of blood vessels [4]. The hemostatic effect is caused by various fractions from the herb and the plant as a whole [5, 6].

It has been established that extracts from the herb of shepherd's purse lower blood pressure, increase intestinal and uterine motility, accelerate blood clotting [1], infusion and liquid

extract in gynecological practice is used for atony of the uterus and as a hemostatic [7]. Preparations of shepherd's purse have both astringent and anti-scorching effect, with diseases of the ureters and malaria. Flavonoids in shepherd's purse have antimicrobial action [8].

In connection with the above, it seems appropriate to standardize the herb of shepherd's purse in terms of the amount of flavonoids.

Materials and methods

At the first stage of the research, the analysis of the spectra of alcoholic extracts and alcoholic extracts after acid hydrolysis from the herb of shepherd's purse (1:50) was carried out. It has been shown that it is inexpedient to determine by the method of direct spectrophotometry based on the position of the absorption maxima of flavonoids due to the superposition of more intense absorption bands of accompanying substances.

When using the spectrophotometric method based on the reaction of complexation with aluminum chloride, a bathochromic shift of the absorption band of flavonoids occurs from 330–350 to 390–410 nm. When an alcoholic solution of aluminum chloride was added, an absorption maximum at 400 nm appeared in the spectrum of extracts, which coincided with the absorption maximum of the spectrum of luteolin with aluminum chloride, and this allows analysis at a given wavelength. The use of a solution of the investigated extraction without adding a reagent to it makes it possible to exclude the influence of colored accompanying substances.

Research results and their discussion

Based on the results obtained (Tables 1 and 2), optimal conditions were selected and a method for determining the amount of flavonoids using luteolin as a standard sample was proposed.

Table 1

Results of determining the effect of extraction conditions on the yield of flavonoids from the herb shepherd's purse

Условия экстракции	Содержание суммы флавоноидов в пересчете на лютеолин, %
Размер частиц сырья, мм	
5–7	0,13
1–2	0,66
Концентрация этанола с 1% HCl, %	
50	0,60
60	0.65
70	0.66
80	0,64
95	0,55
Соотношение сырья и экстрагента	
1:20	0,61
1:30	0,66
1:40	0,66
1:50	0,66
Время экстракции, мин	
60	0,61
90	0,60
120	0,66
150	0,60

Methodology: An analytical sample of the herb's purse is crushed to the size of particles passing through a sieve with a hole size of 2 mm. About 1.25 g (accurately weighed) crushed

herbs are placed in a flask with a thin section with a capacity of 250 ml, 50 ml of ethyl alcohol 70% containing 1% concentrated hydrochloric acid are added, the flask is weighed with an error of ± 0.01 g, connected to a reflux condenser and heated in a boiling water bath for 2 hours. Then the flask is cooled to room temperature, weighed, if necessary, bring its contents to the initial mass with ethyl alcohol 95%.

The contents of the flask are filtered through a funnel with a diameter of 7 cm with a cotton swab inserted with a thickness of not more than 0.5 cm, discarding the first 25 ml of the filtrate (solution A).

In a volumetric flask with a capacity of 25 ml, place 2 ml of solution A, add 5 ml of a 2% alcohol solution of aluminum chloride and bring the volume of the solution to the mark with ethyl alcohol 95% (test solution). After 40 minutes. measure the optical density of the test solution on a spectrophotometer at the absorption maximum at a wavelength of 400 nm in a cuvette with a layer thickness of 10 mm. As a reference solution, a solution is used, consisting of 2 ml of solution A, brought to the mark with ethyl alcohol 95% to the mark in a 25 ml volumetric flask.

table 2

Determination of the reaction conditions of luteolin with aluminum chloride

Условия реакции	Содержание суммы флавоноидов в пересчете на лютеолин
Количество 2%-ного спиртового раствора хлорида алюминия, мл	
1	0,25
2	0,35
3	0,47
4	0,66
5	0,66
Время реакции, мин	
10	0,32
20	0,54
30	0,66
40	0,66
50	0,66

The content of the sum of flavonoids in terms of luteolin and absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = D \cdot 25 \cdot 50 \cdot 100 / 549.41 \cdot m \cdot 2 \cdot (100 - W),$$

where D is the optical density of the solution; 549.41 - specific absorption index of the complex of luteolin with aluminum chloride at a wavelength of 400 nm [9]; m - 1 mass of raw material, g; W is the loss in the mass of the raw material upon drying,%.

The second stage of our research was devoted to the validation of the developed methodology.

The method was validated in terms of linearity, repeatability, reproducibility and correctness of the method [10–12].

Determination of linearity was carried out at 5 concentration levels from the theoretical content of the sum of flavonoids in terms of luteolin in the raw material of the shepherd's purse. The solutions were prepared by diluting the aliquot and increasing the aliquot to measure the quantitative content of the sum of flavonoids in terms of luteolin in solutions having a concentration of 50%, 75%, 100%, 125%, 150%. The criterion for the acceptability of linearity is the correlation coefficient, and if its value is close to one, then the set of data can be described by a straight line. The value of the correlation coefficient must be at least 0.995. The correlation coefficient was 0.9976 (Table 3).

Table 3

Results of the linearity experiment of the method

№ измерения	Содержание, % от нормируемого значения (около)	Концентрация стандартного вещества (лютеолин), мкг/мл	Аналитический отклик (оптическая плотность)
1	50	2,40	0,232
2	75	3,60	0,319
3	100	4,80	0,401
4	125	6,00	0,459
5	150	7,20	0,534

The repeatability of the technique was determined on one sample of raw materials in 6 replicates. The acceptance criterion was expressed as a relative standard deviation, which should not exceed 10%. It was 2.62% (Table 4), which indicates the precision of the technique under repeatability conditions.

Table 4

Quantitative content of the sum of flavonoids in terms of luteolin in raw shepherd's purse

Повторность	Найденное значение, %
1	0,372
2	0,384
3	0,379
4	0,369
5	0,383
6	0,397
Среднее значение	0,381
Относительное стандартное отклонение (RSD), %	2,62

The determination of the intralaboratory reproducibility of the method was carried out on 3 samples in triplicate (performed by 2 chemical engineers) (Table 5). The acceptance criterion was expressed by the value of the relative standard deviation, which should not exceed 15%. It was 5.18%, which indicates the precision of the method in terms of reproducibility.

Table 5

Quantitative content of the sum of flavonoids in terms of luteolin in raw materials shepherd's purse

Повторность	Аналитик	Образец 1, найдено, %	Образец 2, найдено, %	Образец 3, найдено, %
1	1	0,39	0,28	0,31
2	1	0,43	0,30	0,36
3	1	0,39	0,31	0,35
4	2	0,40	0,25	0,31
5	2	0,39	0,27	0,33
6	2	0,40	0,28	0,32
Среднее значение		**0,40**	**0,28**	**0,31**
Относительное стандартное отклонение (RSD), %		**3,87**	**7,63**	**6,36**

The correctness of the method was established by measuring the quantitative content of the sum of flavonoids in terms of luteolin in solutions obtained by adding the required amount of a standard to the test solution for concentrations of 125%, 151%, 168%, 201%. The acceptance criterion was the average% recovery when using solutions of concentrations 125%, 151%, 168%, 201%, corrected by 100% and its average value should be within the following range (100 ± 5)%. It was shown that the% recovery ranged from 99.78 to 102.32%, and its average value was 100.88% (Table 6).

Table 6

Results of experiments with additives

№№ п/п	Найдено, г	Добавлено СО лютеолина, г	Ожидаемое значение, г	Полученное значение, г	Выход, %
1	0,5717	0,1450	0,7167	0,7209	100,59
2	0,5717	0,1450	0,7167	0,7155	99,83
3	0,5717	0,1450	0,7167	0,7223	100,78
4	0,5717	0,2900	0,8617	0,8754	100,43
5	0,5717	0,2900	0,8617	0,8769	101,76
6	0,5717	0,2900	0,8617	0,8739	101,42
7	0,5717	0,3866	0,9583	0,9600	100,18
8	0,5717	0,3866	0,9583	0,9805	102,32
9	0,5717	0,3866	0,9583	0,9711	101,34
10	0,5717	0,5799	1,1516	1,1598	100,71
11	0,5717	0,5799	1,1516	1,1683	101,45
12	0,5717	0,5799	1,1516	1,1491	99,78
Среднее значение выхода, %: 100,88					

Conclusion

In the course of research, it was found that the technique is easily reproducible, affordable, takes a minimum of working time, and does not require expensive reagents. It allows you to objectively assess the quality of herbal medicinal raw materials in shepherd's purse. Analysis of industrial batches of raw materials showed the content of the sum of flavonoids in terms of luteolin, the proposed method ranges from 0.28 to 0.66%, which allows us to propose a rate of content of active substances of at least 0.20%.

The results obtained are included in the FSP 9245-08 of OJSC Krasnogorskleksredstva.

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