Quantification of the amount of flavonoids in clove buds

I.A. Tarrab, O.V. Evdokimova
(GBOU VPO Moscow State Medical University named after I.M.Sechenov, Moscow)

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IA Tarrab, OV Evdokimova
IMSechenov Moscow medical state university (Moscow, Russian

RESUME

The research of developing a methodic for determination of quantity of flavonoids in carnation buds using differential spectrometry as well as results of validation of the methodic are presented. The acceptance criteria for the method were determined. The norm for content of biologically active substances in raw carnation is suggested.

Keywords: carnation bud, quantification, flavonoids

SUMMARY

The article presents studies on the development of a method for the quantitative determination of the amount of flavonoids in clove buds 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 of the content of biologically active substances in the raw material of cloves has been proposed.

Key words: clove buds, quantification, flavonoids.

INTRODUCTION

Modern requirements for regulatory documents imply the mandatory inclusion of the section "Quantitative determination" in the quality standards for a medicinal product.

The aim of our work was to develop a method for the quantitative determination of biologically active substances (BAS) for a promising type of medicinal plant material - clove buds and its validation.

MATERIALS AND METHODS

The objects of the study were 6 industrial batches of clove buds that meet the requirements of GOST 29047-91 [1].

At the first stage of the research, the analysis of the spectra of alcoholic extracts from clove buds (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 spectrophotometric method, founded on reactions 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 405 nm appeared in the spectrum of extracts from clove buds, which coincided with the absorption maximum of the spectrum of hyperoside 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.

RESULTS AND DISCUSSION

Based on the results obtained (Tables 1 and 2), optimal conditions were selected and a method for determining the sum of flavonoids using hyperoside as CRM was proposed.

Methodology. An analytical sample of the buds was ground to a particle size passing through a 1.0 mm sieve. About 1.0 g (accurately weighed) of crushed buds was placed in a flask with a thin section with a capacity of 250 ml, 50 ml of 40% alcohol was added, the flask was weighed with an error of ± 0.01, connected to a reflux condenser and heated in a boiling water bath for 2 hours. ... Then

the flask was cooled to room temperature and weighed, if necessary, brought to the initial weight with 40% alcohol. The contents of the flask were filtered through a folded paper filter, discarding the first 25 ml of filtrate (solution A).

Test solution. In a 25 ml volumetric flask, 2 ml of solution A was placed, 3 ml of a 2% alcohol solution of aluminum chloride was added, and the volume of the solution was adjusted to the mark with 96% alcohol.

Table 1

Results of determining the effect of extraction conditions on the yield of flavonoids from clove buds

Условия экстракции	Содержание суммы флавоноидов в пересчете на гиперозид, %
Размер частиц сырья, мм	
5-7	3,51
1-2	3,77
Концентрация спирта, %	
30	3,24
40	3,77
50	3,19
70	3,02
96	1,99
Соотношение сырья и экстрагента	
1:30	3,83
1:40	3,67
1:50	3,77
1:60	3,73
1:80	3,73
Время экстракции, мин.	
30	3,13
60	3,29
90	3,50
120	3,77
150	3,50
180	3,51

table 2

Results of determining the conditions for the reaction of hyperoside with aluminum chloride

Условия реакции	Содержание суммы флавоноидов в пересчете на гиперозид, %
Количество 2 % спиртового раствора хлорида алюминия, мл	
1	3,62
2	3,77
3	3,77
Время реакции, мин.	
10	3,60
20	3,69
30	3,77
40	3,77
50	3,76

Reference solution. In a volumetric flask with a capacity of 25 ml, 2 ml of solution A was added, then 0.1 ml of concentrated acetic acid was added and the volume was adjusted to the mark with 96% alcohol.

After 40 minutes. measure the optical density of the test solution on a spectrophotometer at the absorption maximum at a wavelength of 405 nm in a cuvette with a layer thickness of 10 mm.

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

X = (A 50 25 100) / 330 a 2 (100-W)),

where:

A is the optical density of the solution;

330 - specific absorption index of the complex of hyperoside with aluminum chloride at a wavelength of 405 nm [8];

a is the mass of raw materials in grams;

W is the moisture content of the raw material in percent.

The method was validated according to the following criteria: linearity, repeatability, intralaboratory reproducibility, and accuracy [2–7].

Determination of linearity was carried out at 5 levels of hyperoside concentrations - 30%, 50%, 100%, 150%, 200%, of the normalized value. The absorbance value (A) was calculated as the average of three measurements.

The criterion for the acceptability of linearity was 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 0.9974 (Table 3).

Table 3

Linearity Test Results

№ измерения	Содержание, % от нормируемого значения (около)	Концентрация стандартного вещества (гиперозид), мкг/мл	Аналитический отклик (оптическая плотность)
1	30	4,35	0,159
2	50	7,25	0,243
3	100	14,50	0,533
4	150	21,75	0,713
5	200	29,00	1,003

Table 4

Repeatability test results

Повторность	Содержание суммы флавоноидов в пересчете на гиперозид, %
1	3,77
2	3,66
3	3,83
4	3,73
5	3,76
6	3,77
Среднее значение	3,75
Относительное стандартное отклонение (RSD), %	1,49

The repeatability of the method was determined on one sample of raw materials in 6 replicates over a short period of time using the same reagents and equipment. The acceptance criterion was expressed as a relative standard deviation, which should not exceed 10%. It was 1.49% (Table 4), which indicates the precision of the technique under repeatability conditions.

The determination of the intralaboratory reproducibility of the method was carried out by 2 chemical engineers on 3 samples in triplicate (Table 5). The acceptance criterion was expressed by the value of the relative standard deviation, which should not exceed 15%. It was 3.77%, which indicates the precision of the technique in terms of reproducibility.

The correctness of the method was established by measuring the quantitative content of the sum of flavonoids in terms of hyperoside in solutions obtained by adding the required amount of a standard to the test solution for concentrations of 139%, 152%, 178%, 203% (four levels). The acceptance criterion was the average% recovery when using solutions of concentrations 139%, 152%, 178%, 203%, corrected by 100%, and its average value should be within 100 ± 5%. It is shown that the% recovery ranged from 98.31% to 104.62%, and its average value was 100.99% (Table 6).

Based on the results obtained, this technique can be considered validated.

Table 5

Method reproducibility test results

Повторность	Аналитик	Содержание суммы флавоноидов в пересчете на гиперозид, %		
		Образец 1	Образец 2	Образец 3
1	1	3,77	3,53	3,69
2	1	3,46	3,61	3,51
3	1	3,83	3,77	3,48
4	2	3,79	3,69	3,55
5	2	3,80	3,66	3,55
6	2	3,82	3,60	3,52
Среднее значение		3,75	3,64	3,55
Относительное стандартное отклонение (RSD), %		3,77	2,27	2,06

Table 6

Results of checking the correctness of the method

№ п/п	Найдено, мг	Добавлено СО гиперозида, мг	Ожидаемое значение, мг	Полученное значение, мг	Выход %
1	0,3771	0,1482	0,5213	0,5270	101,09
2	0,3771	0,1482	0,5213	0,5215	100,04
3	0,3771	0,1482	0,5213	0,5125	98,31
4	0,3771	0,1965	0,5736	0,5719	99,70
5	0,3771	0,1965	0,5736	0,5938	103,52
6	0,3771	0,1965	0,5736	0,6001	104,62
7	0,3771	0,2929	0,6700	0,6780	101,19
8	0,3771	0,2929	0,6700	0,6853	102,28
9	0,3771	0,2929	0,6700	0,6726	100,39
10	0,3771	0,3893	0,7664	0,7702	100,50
11	0,3771	0,3893	0,7664	0,7789	101,63
12	0,3771	0,3893	0,7664	0,7554	98,56
Среднее значение выхода, %: 100,99					

Table 7

Results of determining the content of the sum of flavonoids in terms of hyperoside in the buds carnation

Образцы бутонов гвоздики	Содержание суммы флавоноидов в пересчете на гиперозид, %
Гвоздика цельная, изготовитель ООО «ПК «МЭТР» сер.21.05.2012	3,64
Гвоздика цельная, изготовитель ООО «ПК «МЭТР» сер.06.06.2012	3,64
Гвоздика цельная, сер.21.03.2012	3,56
Гвоздика цельная, изготовитель ООО «Славянский пищекомбинат», сер.01.12.2011	3,52
Гвоздика цельная, изготовитель ООО «Славянский пищекомбинат», сер.02.04.2012	3,83
Гвоздика цельная, изготовитель Котани ГмбХ, партия (L0606141120) 126413	2,67

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 medicinal plant raw materials of cloves. The analysis of industrial batches of raw materials showed that the content of the sum of flavonoids in terms of hyperoside, by the proposed method, ranges from 2.67% to 3.83%. The studies carried out made it possible to propose a standard for the content of biologically active substances of at least

2.50% (Table 7). This technique can be included in the Medicinal Product Quality Standard.

LITERATURE

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Author's address

Ph.D. Evdokimova O.V., Associate Professor, Department of Pharmacy.

oevdokimova2010@mail.ru

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