New Approaches to Standardization of St. John's Wort Herbs

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New approaches to standardization of herb of Hypericum

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RESUME

An express-method for quantitative determination of active substances in herbs of Hypericum perforatum and Hypericum maculatum, harvested in different areas of the Republic of Tatarstan, is developed. Optimal extraction conditions of flavonoids - principal active substances of these herbs, and conditions of their spectrophotometric determination after reaction with aluminum chloride have been determined. The accuracy of the method has been tested statistically, the error of the method does not exceed 0.9%.

It is proposed to use for the analysis of the herb of H. maculatum the specific index of hyperoside (the dominant flavonoid of this plant) and aluminum chloride complex for calculations, and for the herb of H. perforatum - specific absorption index which takes into account the quantitative ratio of the two major flavonoids of this plant - hyperoside and routine.

Keywords: Hypericum perforatum, Hypericum maculatum, flavonoids, hyperoside, rutin, express-method, standardization.

SUMMARY

The aim of the study was to develop an express method for the quantitative determination of active substances in the herb St. John's wort. The tests were carried out on the grass of two types of St. John's wort - perforated and spotted, harvested in different regions of the Republic of Tatarstan. As a result of the experiments, the optimal conditions for the extraction of flavonoids, the main active substances of this raw material, were selected and the conditions for their spectrophotometric determination by the products of the reaction with aluminum chloride were developed. The reliability of the method was checked statistically, the error of the method does not exceed 0.9%.

It is proposed to use the specific index of the complex with aluminum chloride of the dominant flavonoid of this plant, hyperoside, in the analysis of the herb St. John's wort, for calculations, and in the analysis of the herb St.

Key words: St. John's wort and spotted, flavonoids, hyperoside, rutin, express method, standardization.

Introduction

In the Russian Federation, two species of the genus St. John's wort (Hypericum L.) are used - St. John's wort (Hypericum perforatum L.) and St. time, the herb of both types of St. John's wort is used absolutely equally for the preparation of infusions, the production

St. John's wort tincture, is included in the form of extracts in the complex domestic preparations "Prostanorm" and "Sibektan", and is also an integral part of a number of balms and elixirs ("Panta-Forte", "Evalar", "Vivaton", "Altai", "Demidovsky "). In the European Pharmacopoeia, only one type of St. John's wort is official - St. John's wort.

In domestic medicine, it is the group of flavonoids that is considered the most important from the point of view of the therapeutic effects manifested by the herb St. John's wort, and the quality of raw materials is assessed by its content. The Russian Pharmacopoeia (GF XI) [1] standardizes the herb St. John's wort by the content of the sum of flavonoids, while the European Pharmacopoeia - by the content of anthracene derivatives ("total hypericins") [2].

Method GF XI assumes fractional threefold extraction of raw materials with 50% alcohol for 30 minutes each. Subsequently, a solution of aluminum chloride is added to an aliquot of the obtained extracts and the resulting solution is photometric at a wavelength of 412 nm. To calculate the total content of flavonoids in the herb St. John's wort is used

the optical density of the complex of GSO rutin with a solution of aluminum chloride, which is determined in parallel in this method. Unfortunately, in the text of the monograph on the herb of St. John's wort in the State Pharmacopoeia XI, gross misprints were made, in fact, making the technique impracticable. In the regulatory documents published in recent years [3], the pharmacopoeial technique is reproduced, but without typos, in addition, it is proposed to carry out calculations, not only by measuring the absorption index of rutin with aluminum chloride, but also using the specific absorption index of this complex at 415 nm equal to 248. Contents the amount of flavonoids is standardized in raw materials at a level of at least 1.5%. The pharmacopoeial method for the determination of flavonoids in the herb St. John's wort was subjected to critical analysis in the work of Pravdivtseva and Kurkin [4]. The authors concluded

In our opinion, the possibilities for improving the method for determining flavonoids in St. selection of a reference flavonoid, the specific absorption rate of which in combination with aluminum chloride will be used for calculations.

Materials and methods

We used dried samples of St. John's wort and spotted herb harvested in June-July 2011-2012 in the Republic of Tatarstan.

The optical densities of the obtained solutions were determined on a LAMBDA 25 spectrophotometer (Perkin Elmer, United States) in a cuvette with a layer thickness of 10 mm.

HPLC analysis was performed on a Shimadzu LC-20 liquid chromatograph in isocratic mode. A mixture of acetonitrile and 1% glacial acetic acid in the ratio 20:80 was used as a mobile phase; a chromatographic column with a C-18 grafted phase was used as a stationary phase (sorbent granulation 10 μm, size 250 x 4.6 mm). The flow rate of the eluent in the assay was 1 ml / min. Detection was performed at an analytical wavelength of 360 nm.

Results and discussion

The content of flavonoids, determined by the FSP method [3], was 3.96 ± 0.01% in St. John's wort, and 4.65 ± 0.02% in St. John's wort.

Since in the above-mentioned work [4] the concentration of alcohol used for the extraction of flavonoids in the pharmacopoeial procedure was questioned, the choice of alcohol for the extraction of flavonoids was first investigated. The results on the yield of flavonoids from the herb H. perforatum and H. maculatum at different concentrations of ethyl alcohol are given in table. one.

Table 1

The content of flavonoids in the herb H. perforatum and H. maculatum when used for the extraction of ethyl alcohol of various concentrations,%

Grass	Flavonoid yield, (% by weight of dry raw materials) / (% of the total amount in raw materials)		
	50% ethanol	70% ethanol	95% ethanol
H. perforatum	3.96 ± 0.11 / 100	3.89 ± 0.02 / 98	3.78 ± 0.03 / 96
H. maculatum	4.65 ± 0.02 / 100	4.34 ± 0.03 / 93	4.03 ± 0.03 / 8

table 2

The release of flavonoids from the herb H. perforatum and H. maculatum, depending on extraction time with 50% ethanol in a ratio of 1: 100,%

	Flavonoid yield, (% of dry raw material weight) / (% of total amounts in raw materials)			
Extraction time, min	5	10	15	thirty
H. perforatum herb	3.55 ± 0.04 / 89	3.64 ± 0.03 / 91	3.62 ± 0.20 / 93	3.83 ± 0.02 / 97
H. maculatum herb	3.83 ± 0.11 / 82	4.25 ± 0.02 / 91	4.35 ± 0.05 / 94	4.53 ± 0.02 / 97

Table 3

Metrological characteristics of the rapid method for the determination of flavonoids in the herb St. John's wort and spotted

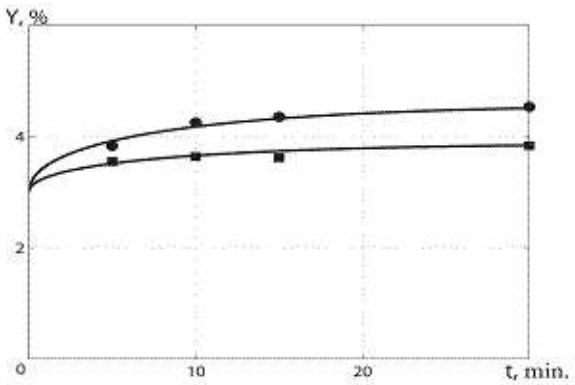
	F	$\overline{X}$	S^2	S	P	t (0,95; 9)	Δx	ε, %
H. perforatum	9	3,62	0,0019	0,0436	0,95	2,26	0,03	0,9
H. maculatum	9	4,65	0,000515	0,0227	0,95	2,26	0,02	0,4

The results obtained indicate that 50% concentration of ethyl alcohol in the studied range is optimal for raw materials of both types of St. John's wort.

At the next stage of research, the dynamics of the yield of flavonoids from raw materials was studied depending on the extraction time at the optimal alcohol concentration. In all cases, a 1: 100 ratio of raw material and solvent was used.

The dependence of the yield of flavonoids on the extraction time was tested in experiments at least in 4 replicates. The results are presented in table. 2.

A graphical version of the tabular data and an adaptation model of the extraction process for H. perforatum and H. maculatum flavonoids are shown in Fig. one.



Rice. 1. Extraction of flavonoids from the herbH. perforatum (■) and H. maculatum (●). Adaptation models (solid line) and experimental results (markers) using 50% ethanol in a ratio of 1: 100 to raw materials. Y-axis - flavonoid yield in% of dry raw material masses; The abscissa is the extraction time, min;

As can be seen from the results obtained, more than 90% of flavonoids from both types of St. John's wort are extracted in 10 minutes. This made it possible to offer us a variant of the express method for the determination of flavonoids in the herb of both types of St. John's wort, using a correction factor for incomplete extraction.

In our proposed express method for the determination of flavonoids in the herb St. John's wort and spotted, the extraction time is reduced to 10 minutes, and to calculate the true content of flavonoids, a correction factor for the incompleteness of flavonoid extraction is introduced equal to 1.1.

Rapid method for the quantitative determination of flavonoids in the herb St. John's wort An analytical sample of raw materials is crushed to a particle size passing through a sieve with holes 1 mm in diameter. About 1 g (accurately weighed) of the crushed raw material is placed in a flask with a thin section with a capacity of 250 ml, 100 ml of 50% alcohol is added, the flask is connected to a reflux condenser and heated in a boiling water bath for 10 minutes from the moment the solvent boils. The flask is then cooled to room temperature under running cold water and filtered through a paper filter into a 100 ml measuring cylinder (solution A).

1 ml of solution A is 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 40 min. measure the optical density of the solution on a spectrophotometer at a wavelength of 415 nm in a cuvette with a layer thickness of 10 mm. The following solution is used as a comparison solution: 1 ml of solution A is placed in a 25 ml volumetric flask, 1 drop of diluted acetic acid is added and the volume of the solution is brought to the mark with 95% alcohol.

The content of the sum of flavonoids in terms of rutin and absolutely dry raw materials in St. John's wort in percent (X) is calculated by the formula:

$$X = \frac{D \cdot V \cdot 25 \cdot 100 \cdot 1{,}1}{248 \cdot m \cdot (100 - W)}$$

where D is the optical density of the test solution; 248 - specific absorption index of the complex of rutin with aluminum chloride at 415 nm; V is the volume of solution A

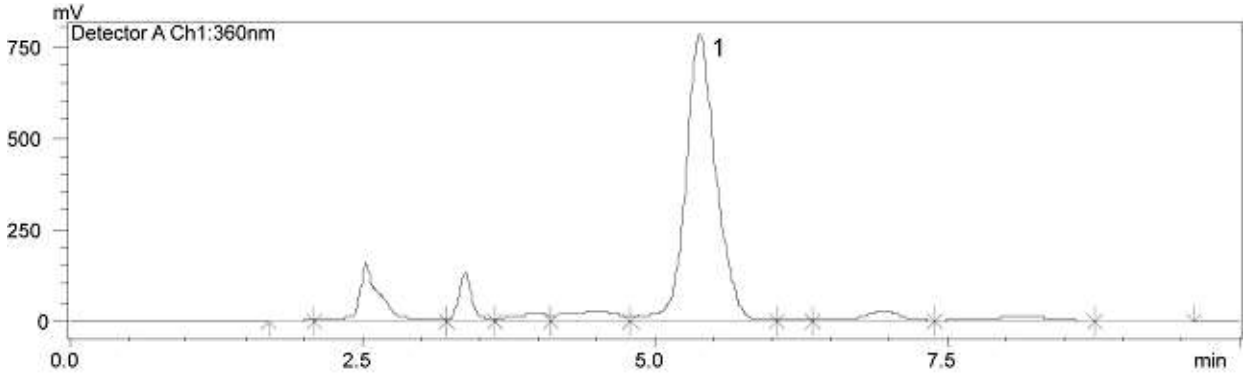
in a graduated cylinder; m is the mass of raw materials in grams; 1.1 - correction factor for incomplete extraction of flavonoids; W - loss in weight on drying of raw materials in percent.

To determine the metrological characteristics of the developed methods, 10 parallel determinations were carried out (Table 3). The results obtained indicate satisfactory reproducibility and correctness of the methods, the error of the method does not exceed 0.9% for the herb St. John's wort, and 0.4% for the herb St. John's wort.

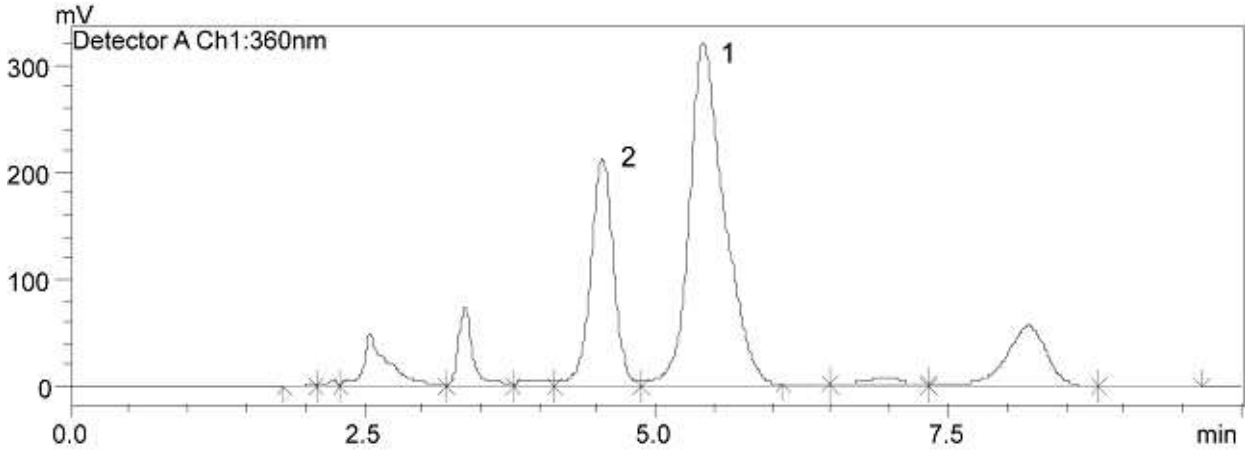
In the method developed by us for the calculations, we used the specific absorption index of the complex of rutin with aluminum chloride at 415 nm, which is the same in the PSP on the herb St. John's wort [3]. In work [5] it was shown that the main flavonoid of the herb H. maculatum is hyperoside, and rutin is practically absent in it. The complex of hyperoside with aluminum chloride at the wavelength used in this technique has a specific absorption index equal to 380, and its use for calculations will lead to a significant change in the results.

To clarify the reference flavonoid, according to the specific absorption index of which with aluminum chloride, calculations of the content of flavonoids should be made, we carried out a study of the qualitative and quantitative composition of flavonoids in both types of St. John's wort by HPLC. The results shown in Fig. 2 and 3 showed that hyperoside is the dominant flavonoid in the herb St. John's wort; rutin is present only in trace amounts. Hyperoside is also the dominant flavonoid in the herb St. John's wort, but rutin is also present in significant quantities (their quantitative ratio is described as 2.6: 1). Thus, for the correct calculation of the content of flavonoids in the herb St. John's wort, it is necessary to use the specific absorption index of the complex of hyperoside with aluminum chloride at 415 nm equal to 380.

Thus, the true values of the content of flavonoids in the studied types of raw materials are, most likely, for the herb H. maculatum at the level of 3.0%, and for the herb H. perforatum at the level of 2.9%, and not 4.65 and 3.96 %, respectively.



Rice. 2. Chromatogram of herbal extractH. maculatum (mobile phase: mixture of 1% a solution of acetic acid and acetonitrile in a ratio of 80:20); 1 - hyperoside.



Rice. 3. Chromatogram of herbal extractH. perforatum (mobile phase: mixture of 1% a solution of acetic acid and acetonitrile in a ratio of 80:20); 1 - hyperoside, 2 - routine.

conclusions

1. The developed express-method for spectrophotometric determination flavonoids in the herb St. John's wort and spotted perforatum allows for determinations with an error not exceeding 0.9%, with a significant reduction in the analysis time.

2. For more accurate results of the content of flavonoids in these types of raw materials, it is necessary for the herb St. John's wort to use the specific absorption index of the complex of hyperoside with aluminum chloride at 415 nm equal to 380, and for St. John's wort - the conditional specific absorption index of the sum of hyperoside and rutin equal to 343.

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