

Development of a method for the quantitative determination of the amount of flavonoids in a homeopathic matrix tincture from fresh shoots of Siberian fir
(*Abies sibirica* Ledeb.)

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The method of total flavonoid content quantitative determination development in the fresh shoots of siberrian fir homoeopathic mother tincture

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RESUME

There was developed the method of the quantitative determination of the total flavonoids in the homoeopathic mother tincture of Siberrian fir calculated on the rutin. The relative error of single determination with 95% confidence probability is $\pm 3.2\%$. Experiments with additions of a standard sample of rutin proved the absence of the systematic error.

Keywords: Siberrian fir, *Abies sibirica* Ledeb., Homoeopathic mother tincture, total flavonoid content, spectrophotometry.

SUMMARY

A spectrophotometric method has been developed for the quantitative determination of the sum of flavonoids in terms of a standard sample of rutin in a homeopathic matrix tincture of Siberian fir. The relative error of a single determination with a 95% confidence level is $\pm 3.2\%$. Experiments with the addition of a standard sample of rutin proved the absence of a systematic error of the method.

Key words: Siberian fir, *Abies sibirica* Ledeb., Homeopathic matrix tincture, sum of flavonoids, spectrophotometry.

INTRODUCTION

In accordance with Federal Law No. 61 "On the Circulation of Medicines" dated 04/12/2010, homeopathic preparations are subject to state registration in the presence of normative documentation (ND) for raw materials, homeopathic matrix tincture (GMN) and drug) [6, 7] and entry into the State Register of Medicines (MP) [7].

Homeopathic monopreparations from freshly harvested Siberian fir (*Abies sibirica* Ledeb.) Were approved for use in homeopathic practice on the territory of the Russian Federation in accordance with the Order of the Ministry of Health of the MP No. 335 dated November 29, 1995 [4], however, our analysis of the domestic ND showed that in the RF are absent

pharmacopoeial monographs (FS) on fresh young shoots of Siberian fir, which are raw materials in homeopathy, and GMN from them.

Our analysis of the homeopathic pharmacopoeias of Germany [10], France [11] and Great Britain [9] made it possible to establish that the raw material of Siberian fir and GMN from it is not included in any of them. Therefore, research on the development of methods for standardizing freshly harvested Siberian fir raw materials and GMN from it is relevant, taking into account the need to harmonize the requirements for medicines and the original medicinal product used in homeopathy and allopathy [2].

As a result of the information and analytical research, we found that the predominant groups of biologically active substances (BAS) in the raw material of Siberian fir are flavonoids and essential oils. As a result of an attempt to isolate essential oil from GMN by the pharmacopoeial method of distillation with water vapor, trace amounts of essential oil were obtained, which were insufficient for reliable determination of its quantitative content. Therefore, as one of the criteria of good quality, we proposed the normalization of the quantitative content of the sum of flavonoids in the studied GMN.

The purpose of this study was the development of methodology for quantitative determination of the amount of flavonoids in the GMN of Siberian fir.

MATERIALS AND METHODS

Freshly harvested young shoots of Siberian fir for obtaining GMN were harvested on the territory of Moscow (in the Botanical Garden of Moscow State University) in June 2009. GMN was prepared at the Scientific Research Center of NO "Professional Association of Naturotherapists" in accordance with method 3 of the general FS "Homeopathic matrix tinctures" [5] (the raw materials contain essential oils and resins, and the loss in mass upon drying is less than 60%).

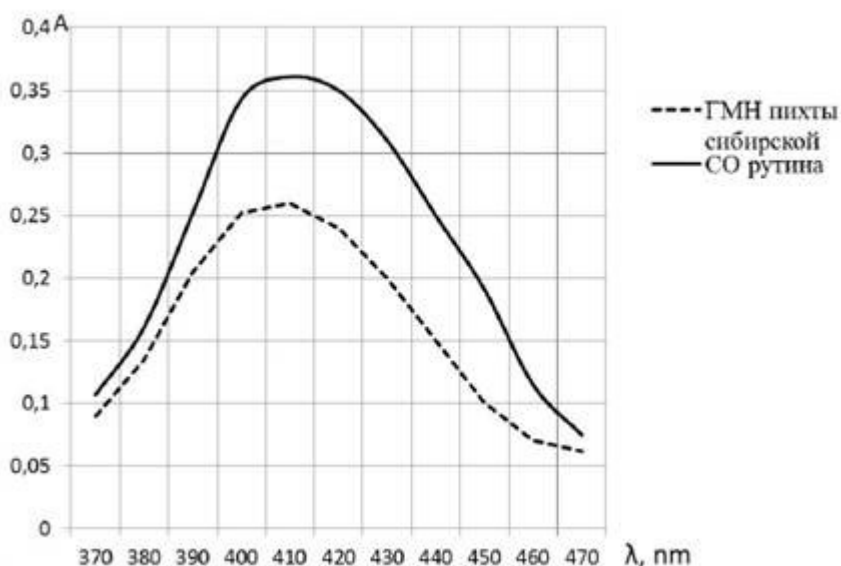
Quantitative content sums of flavonoids identified with using the method of differential spectrophotometry. The measurements were carried out on a U-2800 spectrophotometer (Hitachi, Japan). Rutin from Fluka (catalog no. 84082; the content of the main substance was 90%) was used as a standard sample (SS).

EXPERIMENTAL AND RESULTS

When developing the technique, the complexation reaction with an alcoholic solution of aluminum chloride was used. As a result of the formation of the complex, a bathochromic shift of the first absorption band of the main flavonoids from 330-350 to 390-410 nm is observed. This region is far enough away from the absorption spectra of other phenolic compounds (phenol carboxylic acids, tannins, etc.), which makes it possible to exclude their influence on the quantitative determination of flavonoids. In addition, the use of the differential spectrophotometry method allows the determination of the amount of flavonoids directly in the GMN, since this excludes the influence of the accompanying colored plant substances (chlorophyll, carotenoids, anthracene derivatives

and others) [3, 8].

When developing the method, the optimal conditions for the photometric reaction of complexation of the sum of the flavonoids of the matrix tincture with an alcohol solution of $AlCl_3$ were selected. The following parameters were investigated: the area of maximum light absorption by the complex, the amount of tincture taken for analysis, the concentration and amount of an alcohol solution of $AlCl_3$ required for complete binding of the sum of flavonoids into a complex compound, the dependence of light absorption of photometric compounds on time [8]. When registering the optical density of the products of the complexation reaction at different wavelengths, we obtained the UV spectra shown in Fig. 1.



Rice. 1 Differential absorption spectra of reaction products complexation of the sum of flavonoids HMN of Siberian fir and a solution of CO rutin with 1% alcohol solution of $AlCl_3$...

In parallel, the optical density of the CO complex of rutin with an alcohol solution of $AlCl_3$ was recorded. The region of maximum light absorption of rutin is within 410 ± 2 nm. In fig. 1 shows that the region of maximum light absorption of the complex of the sum of flavonoids in the matrix tincture (409 ± 2 nm) and the maximum absorption of the CO complex of rutin with an alcoholic solution of $AlCl_3$ close. Therefore, in the method of quantitative determination of the sum of flavonoids in the investigated HMN, it was proposed to record the optical density at a wavelength of 410 nm, and the calculation of the percentage of the sum of flavonoids should be carried out in terms of the RS of rutin.

In further studies, the effect of the amount of tincture, concentration and amount of $AlCl_3$ solution was studied on the completeness of the course of the complexation reaction. The results of the study are shown in table. 1–3 (average values of 5 determinations are shown). An aliquot was chosen so that the optical density took a value from 0.3 to 0.5.

Table 1

Influence of the amount of HMN in Siberian fir on the completeness of the reaction complexation (average of 5 determinations)

Количество настойки, мл	Оптическая плотность (D)
0,1	0,128
0,2	0,349
0,3	0,519

table 2

Influence of the concentration of an alcoholic solution of $AlCl_3$ for completeness complexation reactions (average of 5 determinations)

Концентрация раствора $AlCl_3$, %	Оптическая плотность (D)
1	0,355
2	0,351
3	0,348

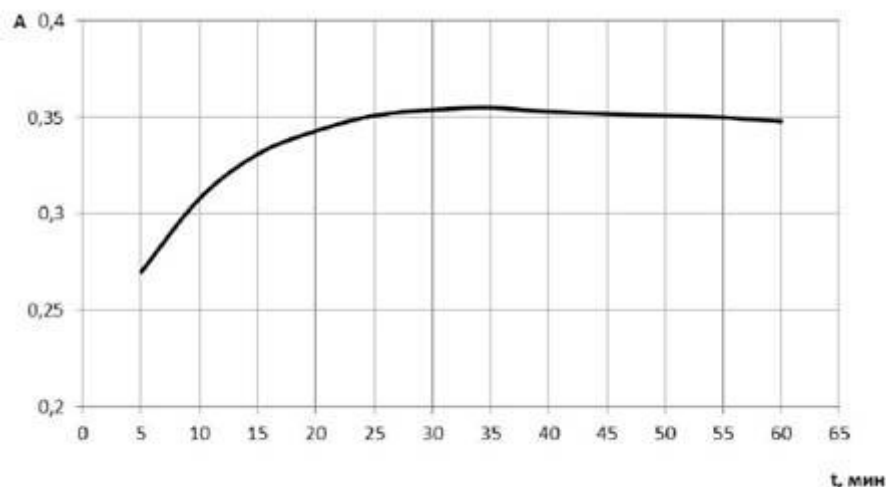
Table 3

Effect of the amount of alcohol solution $AlCl_3$ for completeness complexation reactions (average of 5 determinations)

Количество раствора $AlCl_3$, мл	Оптическая плотность (D)
1	0,368
2	0,349
3	0,358

Based on the data obtained, the optimal conditions for the photometric reaction were selected. It was decided to use 0.2 ml of tincture and 1 ml of 1% $AlCl_3$ solution...

To study the dependence of light absorption on the time of the photometric reaction, the optical density of the solution was recorded every 5 min. Based on the data obtained, a graph is built (Fig. 2).



Rice. 2 Graph of the dependence of optical density on time of photometric complexation reactions of the sum of flavonoids HMN of Siberian fir with 1% alcohol solution $AlCl_3$

The study made it possible to establish that within 25–30 min. the complex is being formed, while the optical density is increasing. Upon further observation, no significant increase in optical density occurs, which corresponds to an almost horizontal section on the graph. Therefore, in the method of quantitative determination of the amount of flavonoids, it is advisable to register the optical density after 30 minutes. after adding the reagent.

Method for the quantitative determination of the content of the sum of flavonoids in GMN of Siberian fir

0.2 ml of the filtered Siberian fir HMN is placed in a 25 ml volumetric flask, 1 ml of 1% aluminum chloride solution is added, the volume of the solution is brought to the mark with 95% alcohol and stirred. After 30 min. measure the optical density of the solution on a spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm. The following composition is used as a comparison solution: 0.2 ml of tincture, one drop of acetic acid are placed in a 25 ml volumetric flask, the solution volume is brought to the mark with 95% alcohol and mixed. In parallel, the optical density of a 0.05% solution of rutin CO is measured, prepared similarly to the test solution with one refinement: 1 ml of rutin CO solution is placed in a 25 ml volumetric flask. The following composition is used as a comparison solution: 1 ml of a 0.05% alcohol solution of rutin is placed in a 25 ml volumetric flask,

Note: Preparation of rutin CO solution. About 0.05 g (accurately weighed) of rutin ("Fluka", catalog No. 84082), pre-dried at a temperature of 130–135 °C for 3 hours, is dissolved in 85 ml of 95% alcohol in a 100 ml volumetric flask while heating in a water bath. Cool down

quantitatively transferred into a volumetric flask with a capacity of 100 ml, bring up to the mark with 95% alcohol and mix. The shelf life of the solution is 1 month.

The content of the sum of flavonoids (X), as a percentage, in terms of CO rutin is calculated by the formula:

$$X(\%) = \frac{D \times m_0 \times V_0 \times 25 \times 100\%}{D_0 \times 100 \times 25 \times V}$$

where:

D is the optical density of the test solution; D₀ - optical density of rutin CO solution; V is the volume of the tincture taken for analysis;

V₀ - volume of rutin CO solution taken for analysis, ml; m₀ is the mass of the sample of CRM rutin, g.

DISCUSSION AND CONCLUSIONS

To check the reproducibility of the developed method, its metrological characteristics were calculated. Determination of the sum of flavonoids was carried out in one laboratory sample of GMN in 7 independent replicates. Metrological characteristics of the method are presented in table. 4.

As can be seen from the data in the table. 4, the relative error of a single determination with a 95% confidence level is ± 3.2%, which indicates the reproducibility of the method. In addition, the relative error in the analysis in triplicate does not exceed ± 1.9%, therefore, it is recommended to carry out the analysis in triplicate in the method.

Table 4

Metrological characteristics of the quantitative determination procedure sum of flavonoids GMN of Siberian fir

\bar{x}	n	f	S \bar{x}	P (%)	t (P;f)	Δx	ϵ , %	ϵ_s , %
0,186	7	6	0,0024	95	2,447	0,0059	±3,2	±1,9

The absence of a systematic error of the method was proved using experiments with addition of CO rutin. The results are presented in table. 5.

Table 5

Results of experiments with addition of CO rutin for GMN of Siberian fir

Определено флавоноидов (в сумме) в образце настойки (мг)	Добавлено рутина (мг)	Суммы флавоноидов (мг)		Ошибка определения	
		должно быть	определено	абс. (мг)	отн. (%)
0,3720	0,0925	0,4645	0,4658	+0,0013	+0,3
0,3720	0,1850	0,5570	0,5749	+0,0179	+3,2
0,3720	0,2780	0,6500	0,6702	+0,0202	+3,1
0,3720	0,3700	0,7420	0,7240	-0,0180	-2,4

Table data. 5 indicate the absence of a systematic error of the method, since the relative error of experiments with additives when analyzing in triplicate is within the error of a single determination and is no more than $\pm 3.2\%$.

So way, us developed methodology quantitative spectrophotometric determination of the sum of flavonoids in the GMN of Siberian fir, based on the complexation reaction of the sum of flavonoids with an alcohol solution of $AlCl_3$... The content of the sum of flavonoids in GMN from Siberian fir raw materials, harvested in Moscow, is $0.19 \pm 0.01\%$ (in terms of RS of rutin). The results obtained show the possibility of their use for the standardization of GMN from freshly harvested Siberian fir raw materials.

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