Federal Technical Regulation and Metrology Agency (Rosstandart)

Technical
Committee TC 450
"Pharmaceuticals"

PHARMACEUTICALS QUALITY ASSURANCE ISSUES

ISSN: 2309-6039
Journal On-line version: **www.humanhealth.ru**

№1 (31) 2021

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ISSN №2309-6039

PI № FS 77-53661
dated April 10, 2013

The Journal is included in the List of peer – reviewed scientific publications, where the main scientific results of thesis for Candidate's Degree, Doctor's Degree should be published according to the letter from the Ministry of Education and Science dated 01.12.2015 № 13-6518.

Address of the editors office: 9, Sharikopodshipnokovskaya str., Moscow, 115088 ROOI "Human Health"
Proof-reader:
Didevich A.V.
Page Make-up:
Ermakova E.V.

Tel.: 8 (495) 674–65–22
8 (926) 917 61 71
E-mail: journal@humanhealth.ru
www.humanhealth.ru

Index in the catalog of Federal Agency on Press and Mass Communications (Rospechat) (Publications of scientific and technical information bodies) in Russia: 57958

Publishing company
ROOI "Human Health"
E-mail: izdat@humanhealth.ru

Printing support:типография
Printing company
"Moscow Print Yard"
Tel.: (495) 7816411, (495) 5455812,
www.printyard.ru

Circulation 3000 copies
Order №2602021

ISSN 2309-6039 №1 (31) 2021

PHARMACEUTICALS QUALITY ASSURANCE ISSUES

JOURNAL OF PHARMACEUTICALS QUALITY ASSURANCE ISSUES

Journal of Research and Practice
Central peer-reviewed publication
A Quarterly Edition. Published since August 2013

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UDC 615.322
https://www.doi.org/10.34907/JPQAI.2021.39.61.001

DEVELOPMENT AND VALIDATION OF METHODS FOR THE QUANTITATIVE DETERMINATION OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES

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The paper presents the results of the development and validation of the method of analysis of arbutin in cowberry leaves by capillary electrophoresis. Sample preparation of the extraction was carried out in accordance with the pharmacopoeial monograph FS.2.5.0063.18 of SP XIV edition. It was found that the UV-spectrophotometry method proposed in the pharmacopoeial monograph determines the quantitative content of not only the arbutin component, but the total amount of phenol-glycosides. The standard content of the arbutin component in the leaves of cowberries by capillary electrophoresis was determined as at least 4%. At the same time, the results of the quantitative content of arbutin are confirmed by using the HPLC method. The method of quantitative determination of arbutin in cowberry leaves by capillary electrophoresis was validated according to the "linearity", "precision" and "intra-laboratory precision", "correctness" parameters. The developed method can be recommended for the analysis of cowberry raw materials.

Keywords: cowberry (*Vaccinium vitis-idaea* L.) leaves, arbutin, capillary electrophoresis, HPLC, UV-spectrophotometry

Currently, there is a tendency to increase the use of chromatographic methods for the analysis of medicinal plant raw materials. In foreign countries, the main method is high-performance liquid chromatography (HPLC), which serves as the basic method of quality control of medicinal plant raw materials. In the pharmacopoeia analysis on the territory of the Russian Federation, 107 individual monographs on medicinal plant raw materials are used, and 7 of which use the HPLC

method to assess the authenticity of raw materials and provide the quantitative determination, a large number of methods are based on electron spectroscopy. This is due to the use of expensive equipment, columns and high-purity solvents (acetonitrile, methanol, etc.) in the HPLC analysis. The spectrophotometric method of analysis is used more often due to the availability of equipment and reagents, but it is worth considering that when choosing this method, we judge the amount of substances in terms of the dominant component. The quantitative content of the major component, in contrast to the HPLC method, cannot be determined by spectrophotometry [1,2].

Capillary electrophoresis (CE) is one of these separation analysis methods that can be used to determine the component composition and the quantitative content of each component. Through the CE, a high efficiency of separation of substances is achieved while the use of expensive reagents and chromatographic columns is not required (the analysis is carried out in a quartz capillary). Thus, it is worth noting the advantages of using CE over HPLC – this is a significantly lower price for a single analysis, the presence of more stable analysis conditions, and the rapidity of the analysis. However, to date, this method has not found the use for the analysis of medicinal plant raw materials.

We have developed a method for the quantitative determination of arbutin by capillary electrophoresis. Previously, the use of capillary electrophoresis for the quantitative determination of arbutin in water extraction from the leaves of cowberry and bearberry was described in the literature [3]. However, we focused on the sample preparation of the extraction of cowberry leaves according to the pharmacopoeial monograph in SP of XIV edition, since the largest amount of arbutin is extracted by 70% ethyl alcohol.

Cowberry (*Vaccinium vitis-idaea* L.) leaves in the SP of XI edition were standardized for the content of arbutin by the method of iodometric titration, in the SP of XIV edition the method of electron spectroscopy is used after preliminary purification of water-alcohol extract using a column with aluminum oxide. The lower limit is standardized as at least 4.5% of arbutin in terms of air-dry raw materials [4].

The purpose of this work is development and validation of methods for quantitative determination of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves by capillary electrophoresis.

MATERIALS AND METHODS

The objects of analysis are cowberry (*Vaccinium vitis-idaea* L.) leaves harvested in various regions of the Russian Federation: Kabardino-Balkar Republic (KBR), Zolsky District, Harbas River bank, autumn 2019; Perm Krai, Kudymkarsky District, spring 2018; Bryansk Region, spring 2018; Irkutsk Region, Bratsky District, Novoe Prirechye village, summer 2019; Altai Krai, Pavlovsky District, autumn 2019; Moscow Region, Mytishchi District, spring 2019. The harvest was carried out in accordance with the requirements of the FS.2.5.0063.18 of SP XIV edition

Sample preparation for determining the content of arbutin in the analyzed objects was carried out in accordance with the requirements of the FS.2.5.0063.18 of SP XIV edition, section "Quantitative determination" [4].

Initially, the electronic spectra of extracts from cowberry (*Vaccinium vitis-idaea* L.) leaves were measured using a SF-2000 spectrophotometer (OKB Spektr JSC, Russia) in the wavelength range from 200 to 400 nm. The calculation was carried out by the value of the specific light absorbance of arbutin.

Further, the content of arbutin was determined by the CE method. To do this, we took the previously obtained extract (FS.2.5.0063.18) that was purified using a column with aluminum oxide and without pre-cleaning. The analysis was

carried out using a Kapel-105M device (Lumex-marketing JSC, Russia) with a quartz capillary (capillary diameter 75 microns, L_c/L_{ef} = 50/60 cm). The quartz capillary was pre-washed sequentially with purified water, 1 M of sodium hydroxide solution, purified water, 1 M of hydrochloric acid solution, purified water and buffer solution. Buffer and washing solutions were filtered through a Vladipor membrane filter of MFAS-B-4 type (STC "Vladipor", Russia), disc diameter 25 mm. The analyzed extract and buffer solution were centrifuged at 8000 rpm, for 5 min. Analysis conditions: sample introduction was carried out hydrodynamically at 150 mbar · s; detection at a wavelength of 254 nm; voltage: +20 kV; capillary temperature: 20°C; electrolyte: 10 mM of borate buffer solution with pH=9.8; analysis time: 10 minutes.

The "Elforan" program (version 3.2.5) was used for processing electrophoregrams. The arbutin content in the extract was calculated using the equation of the calibration graph, which was constructed during the analysis of arbutin reference standard (RS) (Sigma-Aldrich) in the analytical range of concentrations from 0.01 to 0.2 mg/l.

Validation of the method of quantitative determination of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves by capillary electrophoresis was carried out in accordance with SP XIV in terms of linearity, precision (repeatability and intra-laboratory precision) and correctness [3].

To confirm the results, a reversed-phase HPLC variant was used. The analysis was performed using a Steyer chromatograph (Aquilon, Russia) equipped with a Luna C18 column (Phenomenex, USA) with dimensions of 150 × 4.6 mm (sorbent grain size of 5 µm) in the isocratic elution mode. The mobile phase was a mixture of 0.05 M of solution of phosphoric acid and acetonitrile in a ratio of 97:3, the flow rate was 1 ml/min, the volume of the injected sample was 20 µl, the peaks were detected spectrophotometrically at 280 nm. Sample preparation included dilution of 1 ml of crude alcohol extracts from cowberry (*Vaccinium vitis-idaea* L.) leaves with a mobile phase of up to 10 ml. The quantitative content of arbutin in medicinal plant raw materials was determined using the arbutin peak area on chromatograms of reference standard solutions (Sigma-Aldrich, 0.003216% of reference standard solutions in 70% ethyl alcohol).

RESULTS AND DISCUSSION

The spectral analysis of the studied objects showed that the maxima of light absorbance of the extracts coincided with the maximum of light absorbance of arbutin (283±2 nm) (Fig. 1).

The results of quantitative determination of cowberry (*Vaccinium vitis-idaea* L.) leaves from various places of natural vegetation are presented in Table 1.

When studying the optimal conditions for the analysis by capillary electrophoresis, we were faced with the question that arbutin can be present in the raw material in the form of conjugates, for example, in the form of 2-O-caffeoyl arbutin, as well as together with methylarbutin and hydroquinone which is its decomposition product [5]. Some of them can make an important contribution to the spectral method of analysis, since, being phenologlycosides, they are not adsorbed on the column with aluminum oxide

In this regard, the pH of the electrolyte was selected in order to optimally separate the peaks of arbutin and related components. It was found that when using pH=9.6 of 0.01 M borate buffer solution, the best separation of the arbutin peak from other phenoglycosides in the extract is achieved (Fig. 2).

In addition, at pH=9.6, the technique allows us to obtain stable results, since in the pH range of ±0.2, the efficiency of the arbutin peak varies slightly (Table 2).

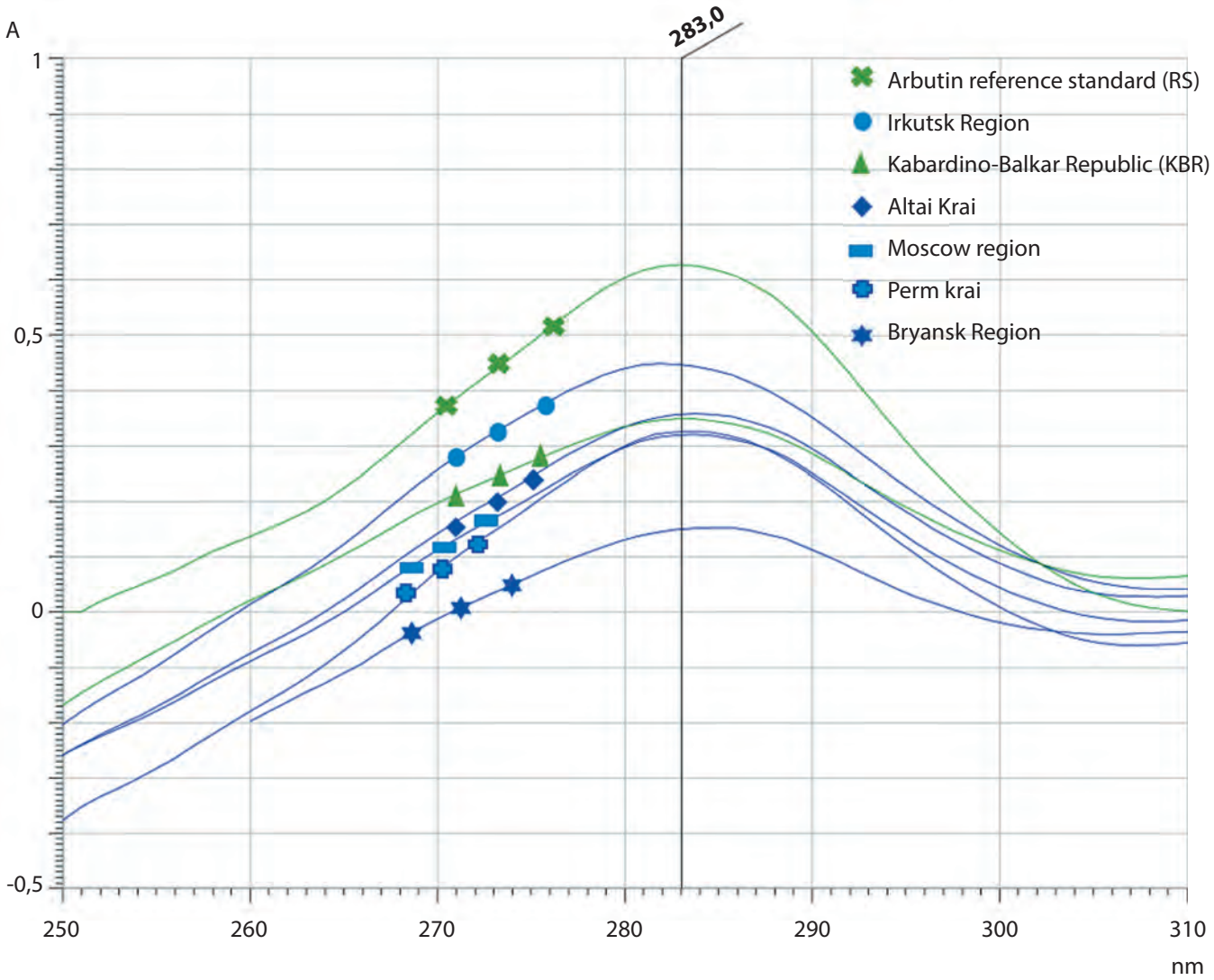

FIG. 1. *Absorption spectra of the solution of arbutin reference standard (RS) and water-alcohol extracts (extraction solvent – 70% ethyl alcohol) from cowberry (Vaccinium vitis-idaea L.) leaves harvested in different places of growth*

Table 1

RESULTS OF QUANTITATIVE DETERMINATION OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES BY UV-SPECTROPHOTOMETRY

Harvesting Region	n	f	$\bar{x}$	S_x	$S_{\bar{x}}$	$t_{(p, f)}$	Δx	ε, %
KBR (autumn 2019)	7	6	8.50	0.1899	0.0718	2.45	0.18	2.07
Perm Krai (spring 2018)	7	6	7.91	0.2566	0.0970	2.45	0.25	3.11
Bryansk Region (spring 2018)	7	6	5.74	0.2093	0.0791	2.45	0.20	3.50
Irkutsk Region (summer 2019)	7	6	11.67	0.5503	0.2080	2.45	0.51	4.36
Altai Krai (autumn 2019)	7	6	8.31	0.2999	0.1134	2.45	0.28	3.34
Moscow Region (spring 2019)	7	6	7.83	0.1907	0.0721	2.45	0.18	2.26

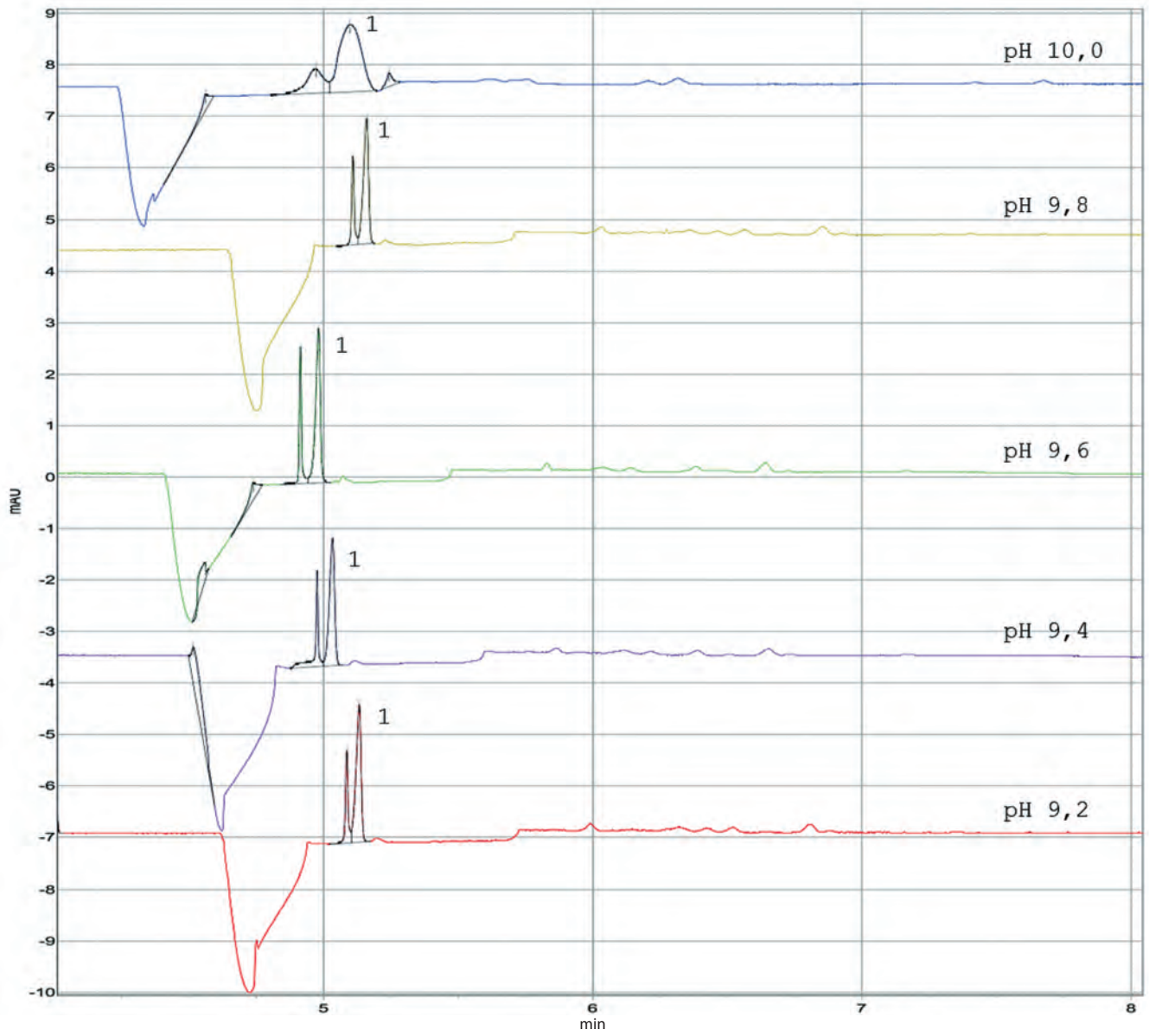

FIG. 2. *Electrophoregrams of water-alcohol extract (extraction solvent – 70% ethyl alcohol) from cowberry (Vaccinium vitis-idaea L.) leaves (Kabardino-Balkar Republic), obtained at different pH values of 0.01 M of borate buffer solution (1 – arbutin)*

Further, according to the method, the extracts obtained by cleaning using a column with aluminum oxide and the crude extracts were analyzed under the selected conditions (Fig. 3)

The difference between the content of arbutin in the crude and purified extract is within the permissible error of the method used, so it is advisable to conduct an analysis by capillary electrophoresis without pre-cleaning the extract from the accompanying phenolic compounds, which will reduce the analysis time.

Table 2

RESULTS OF EVALUATING THE EFFECTIVENESS OF THE ARBUTIN PEAK WHEN USING A BORATE BUFFER SOLUTION WITH DIFFERENT PH VALUES

Effectiveness, thousands of theoretical plates				
pH=9.2	**pH=9.4**	**pH=9.6**	**pH=9.8**	**pH=10.0**
414	418	434	439	18

Thus, we have proposed the following scheme for obtaining and analyzing the extract of cowberry (*Vaccinium vitis-idaea* L.) leaves for the content of arbutin by capillary electrophoresis: 0.5 g of raw materials (exact sample weight) with a particle size of 1 mm is extracted with 100 ml of 70% ethyl alcohol, after weighing a flask with a sample and an extraction solvent with an error of ±0.01 g, in a boiling water bath with a backflow condenser for 45 minutes. After extraction, the extract is cooled, the flask is weighed and, if necessary, brought to the original mass with 70% ethyl alcohol. The extract is filtered through a paper filter moistened with 70% ethyl alcohol, discarding the first 10 ml of the filtrate. Then the 3 ml volume aliquot of the resulting filtrate is introduced into a 25 ml volumetric flask and brought to the mark with 70% ethyl alcohol, mixed. Next, 1 ml of extract is centrifuged at 8000 rpm for 5 minutes

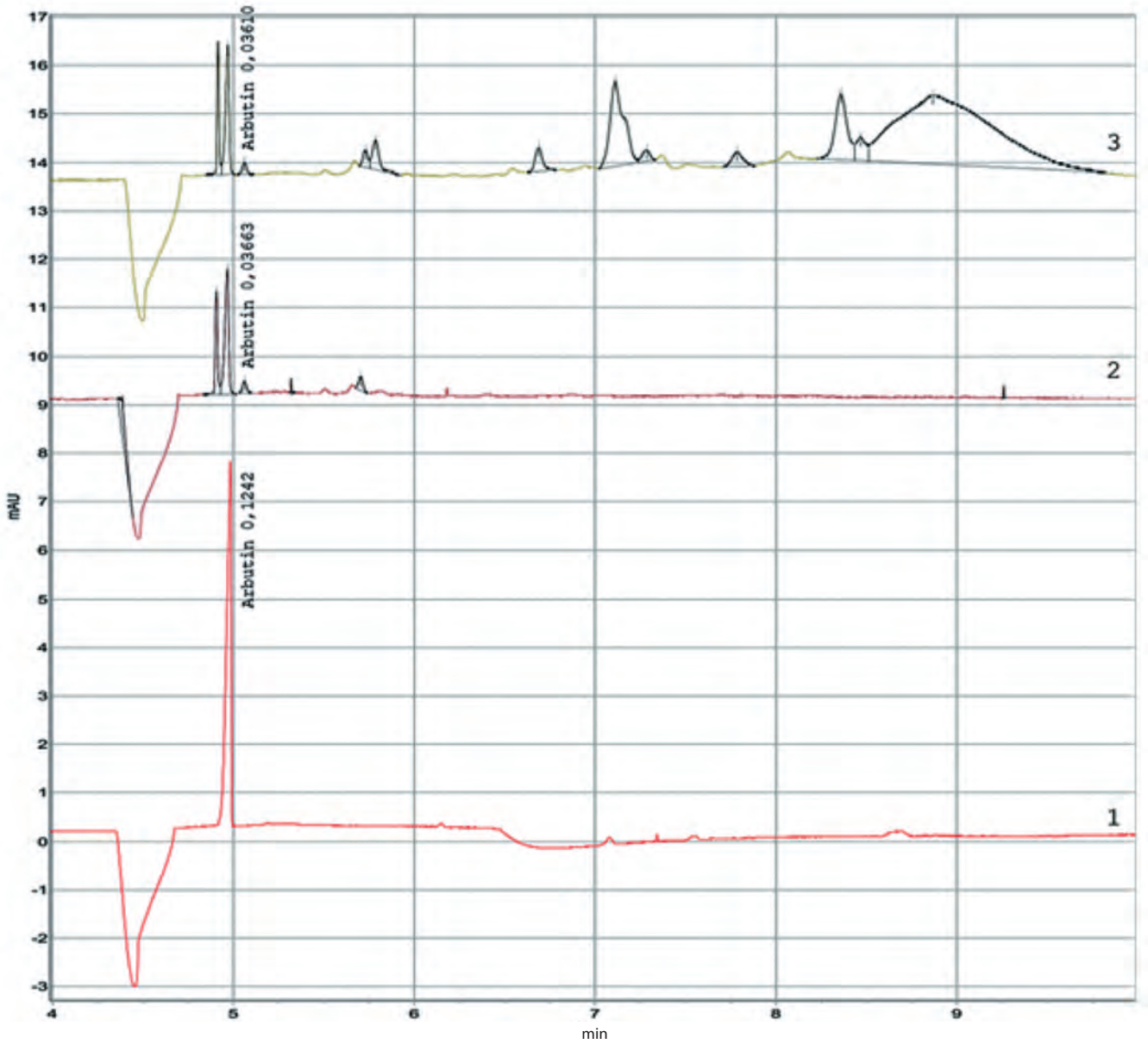

FIG. 3. *Electrophoregrams: 1 – solution of arbutin reference standard (RS); 2-purified water-alcohol extract (extractant – ethyl alcohol 70%) from common lingonberry leaves (Kabardino-Balkar Republic); 3 – crude water-alcohol extraction (extraction solvent – 70% ethyl alcohol) from cowberry (Vaccinium vitis-idaea L.) leaves (Kabardino-Balkar Republic). Arbutin concentration, mg/ml*

and analyzed according to the selected conditions.

The content of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves in terms of air-dry raw materials is calculated by the equation:

$$X, \% = \frac{C_{\frac{mg}{ml}} \times 100 \times 25 \times 100 \times 100}{1000 \times a \times 3 \times (100 - W)} \quad (1),$$

where $C_{\frac{mg}{ml}}$ – the concentration of arbutin in the extract, calculated according to the calibration graph (Fig. 4); a – raw material weight, g; W – raw material moisture, %.

The developed methodology was further subjected to validation evaluation according to the GPM "Validation of analytical methods" [4].

The linearity of the method was determined by the linear nature of the dependence of the analytical signal (peak area) on the concentration of the reference standard of arbutin in solution in the concentration range of 0.083–0.01 mg/ml.

A linear dependence was observed in the analytical range of arbutin concentrations from 0.08 to 0.01 mg/ml, the correlation coefficient was 0.999, which meets the requirements.

The "precision" parameter was evaluated as repeatability (convergence).

The repeatability of the method was evaluated in 7 repetitions conducted by one analyst on one day, using one device and using the same reagents (Table 4).

The relative standard deviation was determined, which should not exceed 10% [6,7]. This value does not exceed 2.7%.

Intra-laboratory (intermediate) precision was determined in one laboratory, but on different days and by two executors for 3 samples of raw materials in 3 repetitions (Table 5).

The relative standard deviation should not exceed 10% [6,7]. In this case, it did not exceed 4.64%. The resulting data show that the method corresponds to the "precision" parameter.

The analytical method is considered as correct if the results of the experiment are within

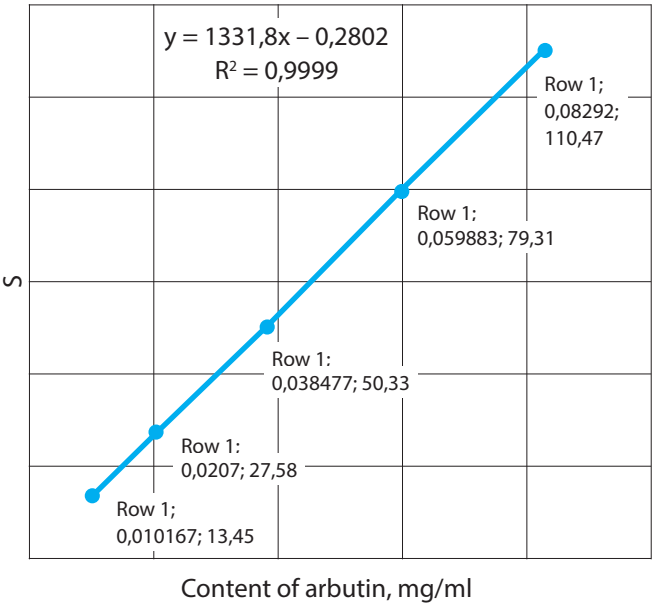

FIG. 4. *Calibration graph of the linear dependence of the peak area of the reference standard of arbutin on its concentration in solution*

the confidence interval of the average result obtained by the method to be validated. The Student coefficient is also calculated and the value of this coefficient should not be greater than the table value for the corresponding sample size ($t_{cal} < t_{tab}$). The correctness was determined by adding a known concentration of a reference standard of arbutin to the test extract obtained from a 0.3 g sample of raw materials with an arbutin content of 60% of the total concentration in the raw material, up to an arbutin content of 80%, 100% and 120%. The ratio of the resulting content

Table 3

THE RESULTS OF THE EVALUATION OF THE "LINEARITY" PARAMETER IN THE VALIDATION OF THE METHOD OF QUANTITATIVE CONTENT OF ARBUTIN IN COWBERRY (VACCINIUM VITIS-IDAEA L.) LEAVES BY CAPILLARY ELECTROPHORESIS

f	$\bar{x}$	$\bar{y}$	b	a	R
4	0.0424	56.23	1331.8	0.2802	0.999

Table 4

RESULTS OF THE EVALUATION OF THE "REPEATABILITY" PARAMETER IN THE VALIDATION OF THE METHOD OF QUANTITATIVE CONTENT OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES BY CAPILLARY ELECTROPHORESIS

Experiment No.	Sample weight, g	Arbutin peak area, mAU·s	Content of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves, %	Metrological characteristics
1	0.5020	51.64	6.99	$\bar{x} = 6.73\%$ $S = 0.1938$ $S_{\bar{x}} = 0.0733$ $\Delta\bar{x} = 0.18$ $\bar{x} \pm \Delta\bar{x} = 6.73 \pm 0.18$ $\bar{\varepsilon} = \pm 2.67\%$
2	0.5015	49.25	6.67	
3	0.5034	48.53	6.55	
4	0.5004	49.89	6.77	
5	0.5011	51.55	7.00	
6	0.5021	48.53	6.57	
7	0.5022	48.51	6.56	
Raw material moisture – 6.26%				

Table 5

RESULTS OF THE EVALUATION OF THE "INTRA-LABORATORY PRECISION" PARAMETER IN THE VALIDATION OF THE METHOD OF QUANTITATIVE CONTENT OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES BY CAPILLARY ELECTROPHORESIS

Content of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves, %			
Repeat	Sample 1 (Irkutsk Region, summer 2019)	Sample 2 (KBR, autumn 2019)	Sample 3 (Altai Krai, autumn 2019)
Executor 1			
1	9.42	6.99	7.17
2	10.10	6.67	6.77
3	10.57	6.55	6.52
Executor 2			
4	9.74	6.77	6.37
5	10.30	6.99	6.73
6	9.50	6.57	6.40
Average value	9.94	6.76	6.66
Standard (relative) deviation (RSD %)	4.64	2.91	4.49

to the introduced content and expressed as a percentage (recovery), the average value of recovery, standard deviation, and relative standard deviation were calculated. The content of arbutin in the test solution was 0.0367 mg/ml (36.7 μg /ml) (Table 6).

So, $t_{cal} < t_{tab}$ (P, f), since 0,73<2,31. The method is correct, because it is not burdened with a systematic error.

As a result, it was experimentally proved that the developed method is suitable for the analysis of arbutin by capillary electrophoresis. The developed method was used for the quantitative analysis of the arbutin content in the samples of cowberry (*Vaccinium vitis-idaea* L.) leaves harvested in several regions of the Russian Federation (Table 7).

As one can see from the results in Table 7, the lowest content of arbutin was found in raw materials harvested in the Bryansk region (4.13±0.18%), the maximum is in the Irkutsk region (9.86±0.43%). Based on the results, the limit of the content of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves is not less than 4.0%, which is slightly lower than in the pharmacopoeial monograph of XIV edition. In this regard, we recommend setting a content standard of at least 4%.

The results show that when using UV spectrophotometry, the results are on average 25% higher (the difference in results is from 16% to 28%) than when determining the quantitative content of the arbutin component by the CE method. Apparently, the contribution of auxiliary compounds in the analysis by UV spectroscopy is anticipated. The wide range of results obtained by UV spectroscopy and capillary electrophoresis (16–28%) may be due to the time of harvesting the raw cowberries (before flowering and after fruit maturation),

Table 6

CALCULATED DATA FOR THE "CORRECTNESS" PARAMETER IN THE VALIDATION OF THE METHOD OF QUANTITATIVE CONTENT OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES BY CAPILLARY ELECTROPHORESIS

Content of arbutin in extract from cowberry (*Vaccinium vitis-idaea* L.) leaves, μg/ml	Added the reference standard (RS) of arbutin		Expected content, μg	Resulted content, μg	Recovery, %	Metrological characteristics
	μl	μg				
22.17	66.6	8.53	30.7	30.81	100.36	R = 100.87 SD = 3.58 RSD = 3.55 t_{cal} = 0.73
22.17	66.6	8.53	30.7	30.84	100.46	
22.17	66.6	8.53	30.7	30.22	98.44	
22.17	124.4	15.93	38.1	38.88	102.05	
22.17	124.4	15.93	38.1	38.04	99.84	
22.17	124.4	15.93	38.1	38.2	100.26	
22.17	189.3	24.23	46.4	46.07	99.29	
22.17	189.3	24.23	46.4	47.31	101.96	
22.17	189.3	24.23	46.4	48.81	105.19	

Table 7

THE QUANTITATIVE CONTENT OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES HARVESTED IN VARIOUS PLACES OF VEGETATION, DETERMINED BY CAPILLARY ELECTROPHORESIS

Place of vegetation	n	f	$\bar{x}$	S	$S_{\bar{x}}$	t (P, f)	$\Delta\bar{x}$	$\bar{\varepsilon}$
KBR (autumn 2019)	7	6	6.73	0.1971	0.0745	2.45	0.18	2.71
Perm Krai (spring 2018)	7	6	5.83	0.1615	0.0611	2.45	0.16	2.66
Bryansk Region (spring 2018)	7	6	4.13	0.1365	0.0516	2.45	0.13	3.17
Irkutsk Region (summer 2019)	7	6	9.86	0.4689	0.1772	2.45	0.43	4.40
Altai Krai (autumn 2019)	7	6	6.61	0.3021	0.1142	2.45	0.28	4.23
Moscow Region (spring 2019)	7	6	5.97	0.2288	0.0865	2.45	0.21	3.55

which regulates the RD, due to geographical factor and other conditions.

In order to confirm the results obtained using the CE, the same raw material sample (KBR, autumn 2019) was analyzed by HPLC. A typical chromatogram is shown in Figure 5, and a chromatogram of a solution of a standard arbutin sample is shown in Figure 6.

The results of the quantitative determination of the same sample of medicinal raw materials by HPLC, performed in six-fold repetition, are presented in Table 8.

The content of arbutin in the analyzed sample of raw materials was 6.48±0.12%, which is comparable to the results obtained by the CE method.

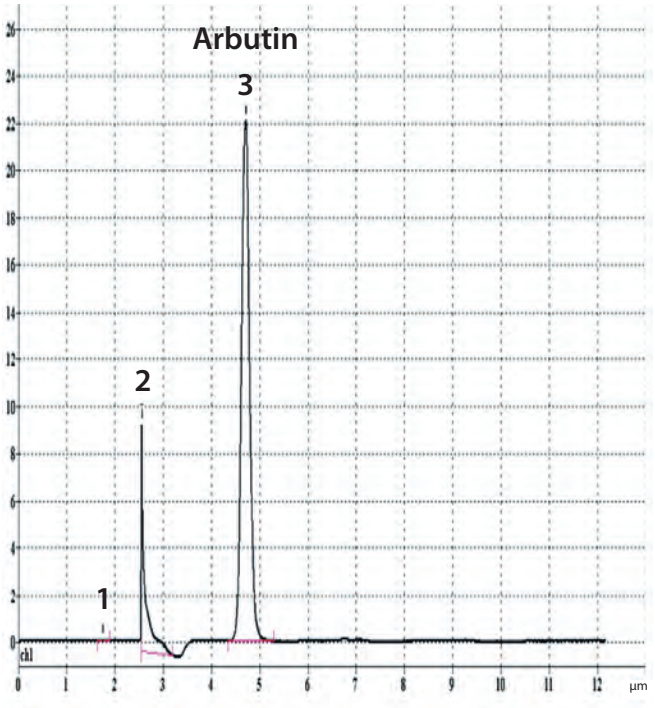

FIG. 5. *Chromatogram of alcohol extraction from cowberry (Vaccinium vitis-idaea L.) leaves*

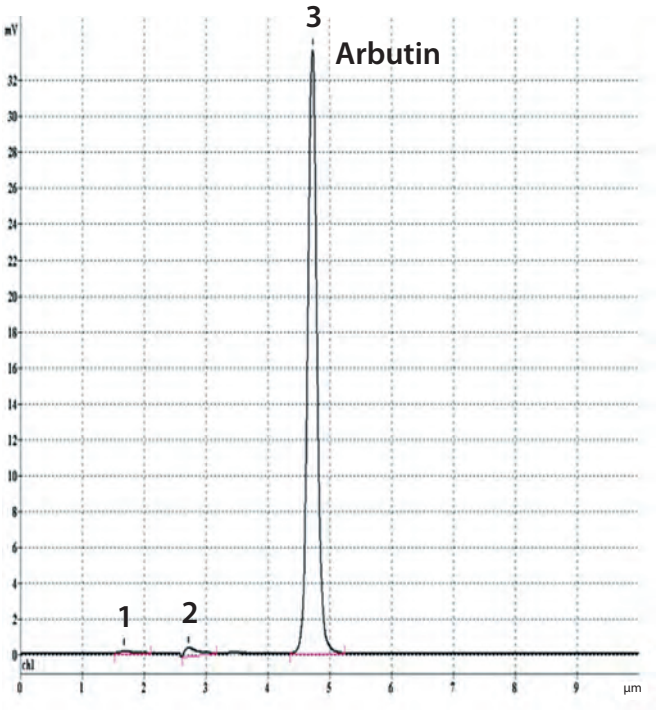

FIG. 6. *Chromatogram of the reference standard of arbutin*

Table 8

RESULTS OF DETERMINATION OF THE QUANTITATIVE CONTENT OF ARBUTIN IN COWBERRY (*VACCINIUM VITIS-IDAEA* L.) LEAVES BY HPLC METHOD

Experiment No.	Sample weight, g	Arbutin peak area, mV*sec	Content of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves, %	Metrological characteristics
1	0.5020	251.16	6.32	$\bar{x} = 6.48\%$ $S = 0.1109$ $S_{\bar{x}} = 0.0453$ $\Delta\bar{x} = 0.12$ $\bar{x} \pm \Delta\bar{x} = 6.48 \pm 0.12$ $\bar{\varepsilon} = \pm 1.8\%$
2	0.5015	253.97	6.39	
3	0.5004	262.63	6.63	
4	0.5034	260.49	6.53	
5	0.5011	259.08	6.53	
6	0.5021	256.88	6.46	
Raw material moisture – 6.26%; arbutin peak area (0.003216% reference standard solution) 253.97 mB · sec				

CONCLUSION

The content of arbutin in cowberry (*Vaccinium vitis-idaea* L.) leaves from various vegetation sites was determined by the spectrophotometric method. The arbutin content ranged from 5.74% to 11.67%.

The arbutin content determined by capillary electrophoresis varied from 4.13% to 9.86%, which is also confirmed by HPLC data. It was found that overestimated results compared to the separation methods were obtained by the pharmacopoeia method such as UV spectrophotometry. Apparently, this is due to the fact that the UV-spectrophotometry method determines the content of not only the arbutin component, but the total amount of phenol-glycosides. The standard content of arbutin in the cowberry (*Vaccinium vitis-idaea* L.) leaves is determined as at least 4%.

Validation of the method of standardization of cowberry (*Vaccinium vitis-idaea* L.) leaves by capillary electrophoresis was carried out. It was found that the method is linear (R<0.99), precise (RSD<4.64%), and not burdened with a systematic error – the Student's criterion t is $t_{cal} < t_{tab}$ (P, f).

The developed method can be recommended for the analysis of cowberry raw materials.

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UDC 615.322
https://www.doi.org/10.34907/JPQAI.2021.71.68.002

ANTIOXIDATIVE ACTIVITY OF *RUBUS IDAEUS* (L.) ONE-YEAR SHOOTS

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The purpose is to study the phenolic compounds and antioxidant activity of the dry aqueous extract of the Rubus idaeus (L.) one-year shoots. The objects of the study were samples of one-year shoots of red raspberry harvested on the territory of the Ilyinsky district of the Perm Region in the herbaceous-spruce forest in June 2019. Young shoots up to 30 cm long were cut and dried with a shade drying method.

The analysis of phenolic compounds was carried out with an Agilent 1100 Series HPLC liquid chromatograph. To determine the antioxidant activity, a reaction with a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used. As a result of a chromatographic study, 12 substances of a phenolic nature were identified in the dry aqueous extract of red raspberry one-year shoots. The dry extract of raspberry shoots contains in the greatest amount – ellagic, benzoic, vanillic acids, vanillin and the flavonoid hyperoside. Dry aqueous extract of raspberry shoots shows pronounced antioxidant activity (IC_{50} – 43.86 μg/ml). This indicator exceeds the activity of the of the sweet-brier fruits infusion in terms of dry residue (IC_{50} – 76.75 μg/ml), but is inferior in terms of the antioxidant effect of the ascorbic acid substance (IC_{50} – 9.46 μg/ml). Dry aqueous extract of raspberry one-year shoots can be recommended for further studies of antioxidant and other types of pharmacological activity.

Keywords: red raspberry, one-year shoots, dry aqueous extract, phenolic compounds, antioxidant activity

The etiology of many cardiovascular and oncological diseases is associated with disorders that occur as a result of the exposure to body cells of free radicals, which are ions or molecules that have an unpaired electron on the valence orbital (peroxyl radical, superoxide anion radical). Most of the body's structures (nucleic acids, proteins, fats and carbohydrates) are potential targets for free radical damage [1].

Many researchers point to the dominant role of phenolic compounds in the development of the antioxidant activity of medicinal herbs [2–5]. Some studies of *Rubus* species show a correlation between the content of phenolic compounds and the level of antioxidant activity [6].

The official medicinal plant raw materials of red raspberries are fruits, but the leaves accumulate a greater amount of phenolic compounds, including polyphenols, which allow you to show antioxidant activity higher than that of fruits [7].

The N-butanol fraction of the ethanol extract of the shoots of *Rubus parvifolius* L. (small-leaved raspberry) has a pronounced hepatoprotective effect against carbon tetrachloride (CCl_4) – induced liver damage, which is partly due to the strong antioxidant ability of the extract [8].

Among the most interesting classes of substances which are responsible for the development of antioxidant action in the shoots and leaves of species of the *Rubus* genus, there are ellagic acid derivatives and flavonoids.

Ellagotanins of *Rubus* species also have high antioxidant activity. The structures of ellagotanins are characterized by the presence of several hydroxyl groups in the ortho position, which exhibit the ability to give off a hydrogen atom [6].

The Purpose of work – research of phenolic compounds and antioxidant activity of dry aqueous extract of *Rubus idaeus* (L.) one-year shoots

MATERIALS AND METHODS

The objects of the study were samples of young green one-year shoots of red raspberry – *Rubus idaeus* (L.). Raspberry shoots were harvested on the territory of the Ilyinsky district of the Perm Territory in spruce forest in June 2019. Young one-year shoots with a length of up to 30 centimeters were cut and dried with a shade drying method.

The extract of dry aqueous shoots of red raspberry was obtained by the following method: a sample weight of air-dried raw materials was placed in a round-bottomed flask, overwatered with water purified in a ratio of 1:30, and extracted with constant stirring for an hour and a half at temperature of 80°C. The solvent cake was separated by filtration and the extract was evaporated with a rotary evaporator.

Three times the amount of 95% ethyl alcohol was added to the evaporated residue, as a result of which the water-soluble polysaccharide complex precipitated. Polysaccharides were separated by filtration through a paper filter, washed out with 80% ethyl alcohol. The extract obtained after separation of polysaccharides was evaporated using a rotary evaporator. The evaporated residue was placed in a drying cabinet and dried at a temperature of 55°C.

The analysis of phenolic compounds was carried out using an Agilent 1100 Series HPLC liquid chromatograph complete with a two-solvent feed and degassing system, a diode-matrix detector, a column thermostat, and an automatic sample input device (autosampler). Software – Agilent ChemStation Rev. A. 09. 03, a column – Atlantis dc18, 100A, 5 microns, 4.6×250 mm. A 0.1% formic acid solution, methanol/acetonitrile (25:75), was used as a mobile phase. The elution mode is gradient, the column temperature is 35°C, the flow rate of the mobile phase is 0.8 ml/min and the volume of the injected sample is 20 µl.

To determine the antioxidant activity, a reaction with a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used [9]. To 1 ml of the test solution, 3 ml of DPPH solution in 95% ethyl alcohol with a concentration of 5 mg/100 ml was added. Next, the antioxidant activity and radical uptake were calculated using the formula:

$$\mathbf{AOA\ (\%) = (A_{Control} - A_{Sample}) / A_{Control} \times 100.}$$

We also determined the value of IC_{50} – the concentration of the substance that can bind half the concentration of the DPPH radical, determined by the inhibition curve obtained when plotting the inhibition graphs as a percentage of the concentration of the substance. As a reference substance, ascorbic acid and a decoction of rosehip fruits were used, since rosehip is a vitamin raw

material and accumulates ascorbic acid and other antioxidants. A decoction of rosehip fruits was prepared in accordance with GPM 1.4.1.0018.15. "Infusions and decoctions" [10].

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RESULTS AND DISCUSSIONS

Many phenolic compounds take an active part in redox processes and promote the binding of reactive oxygen intermediate. Therefore, the content of phenolic compounds in plant extracts is an important indicator that characterizes the possibility of antioxidant and anti-radical effects.

The results of determining the content of phenolic compounds in the extract of raspberry shoots are presented in Table 1.

Table 1 shows that among the identified substances of a phenolic nature, the ellagic acid is contained in the largest amount in the dry extract of raspberry shoots. The content of benzoic acid, vanillinic acid, vanillin and hyperoside is also high.

Some studies suggest that the active metabolite of ellagic acid, urolitin A, can cause neuroprotective effects and protect neurons from oxidative damage [11].

The antioxidant effect of ellagic acid and its metabolites can prevent some cardiovascular diseases associated with oxidative stress by affecting the molecular mechanisms of the vascular bed [12].

In one of the studies in an experiment in mice with type 2 diabetes after administration of vanillin acid for 8 weeks at a dose of 50 mg/kg, a significant decrease in fasting blood glucose

Table 1

CONTENT OF PHENOLIC COMPOUNDS IN THE EXTRACT OF RASPBERRY SHOOTS

Substance	Wave-length (nm), λ	Retention time (min), RT	Content, %
Vanillic acid	254	25.0	0.353
Ellagic acid	254	30.0	0.669
Benzoic acid	254	36.3	0.399
Gallic acid	280	13.3	0.030
Vanillin	280	30.0	0.343
Caffeic acid	300	25.8	0.193
P-coumaric acid	300	30.8	0.042
Ferulic acid	300	32.6	0.092
Salicylic acid	300	38.1	0.074
Hyperoside	350	31.4	0.221
Quercetine	350	43.8	0.054
Kaempferol	350	48.5	0.023

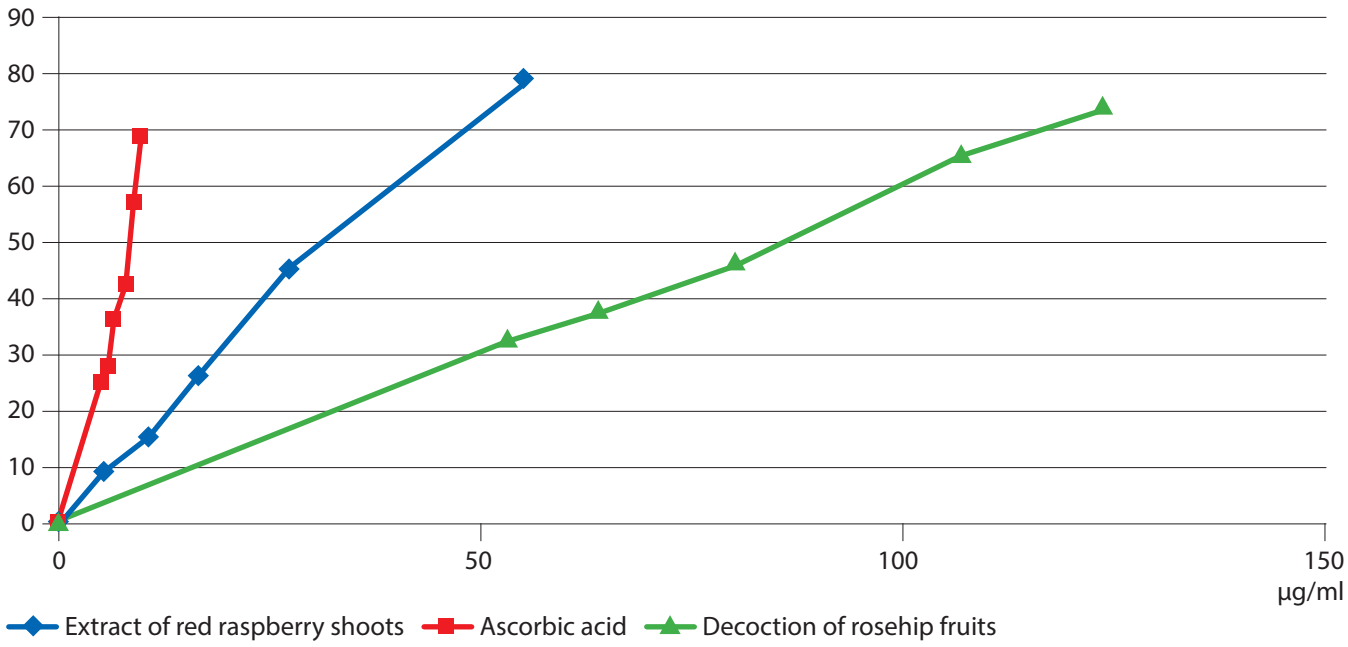

FIG. 1. *Antioxidant activity (%) of dry water extract of first-year raspberry shoots*

and blood pressure was observed in comparison with the control group. Also, in animals treated with vanillinic acid, markers of lipid peroxidation were reduced, which suggests that vanillinic acid helps to combat the oxidative stress [13]. Vanillic acid isolated from the leaves of *Rubus chingii Hu* showed pronounced antioxidant properties [14].

It was interesting to study the antioxidant activity of an aqueous dry extract of first-year raspberry shoots. The results of the determination are shown in Fig. 1 and in Table 2.

Figure 1 shows the dependence of antioxidant activity on the concentration of the studied substances. For red raspberry shoot extract in the concentration range from 5 to 55 μg/ml, the monotonic and spur increase in activity from 10 to 80% is observed. For the reference substance, which was ascorbic acid, in the concentration range from 5 to 11 μg/ml, a spur increase in activity from 0 to 70% is characteristic.

As a result of the study, it was found that the dry water extract of *Rubus idaeus* shoots is able to exhibit pronounced antioxidant activity (Table 2). To correlate the results with the action of known antioxidants, we simultaneously analyzed the substance of ascorbic acid. The IC_{50} for the ascorbic acid substance was found to be

Table 2

ANTIOXIDANT ACTIVITY OF DRY WATER EXTRACT OF THE RED RASPBERRY (*RUBUS IDAEUS* L.) ONE-YEAR SHOOTS (IC_{50})

Sample	Antioxidant activity, IC_{50}, μg/ml
Dry water extract of the red raspberry one-year shoots	43.86±10.06*
Ascorbic acid	9.46±0.93**
Decoction of rosehip fruit	76.75±14.62

** In relation to raspberry shoot extract (t-test) – Student t-test p<0,05*

*** In relation to the decoction of rosehip fruits (t-test) – Student t-test p<0,05*

9.46 µg/ml. Thus, 1 gram of dry water extract of *Rubus idaeus* shoots corresponds to 0.14 g/ equivalent of ascorbic acid. The infusion of rosehip fruits in terms of dry residue shows antioxidant activity almost 2.5 times lower than the studied extract of red raspberry shoots

CONCLUSIONS

As a result of the study, it was found that the dry water extract of *Rubus idaeus* shoots is able to exhibit pronounced antioxidant activity (Table 4). To correlate the results with the action of known antioxidants, we simultaneously analyzed the substance of ascorbic acid. The IC_{50} for the ascorbic acid substance was found to be 9.46 mcg/ml. Thus, 1 gram of dry water extract of *Rubus idaeus* shoots corresponds to 0.14 g/ equivalent of ascorbic acid. The infusion of rosehip fruits in terms of dry residue shows antioxidant activity almost 2.5 times lower than the studied extract of raspberry shoots

As a result of the study, 12 substances of a phenolic nature were identified. The dry extract of raspberry shoots contains ellagic acid in the largest amount. The content of benzoic acid, vanillinic acid, vanillin and hyperoside is also high.

Dry water extract of red raspberry one-year shoots shows a pronounced antioxidant activity, exceeding the activity of the infusion of rosehip fruits, but several times inferior in activity to the reference substance, which was ascorbic acid. Thus, the one-year shoots of red raspberry can be considered as a source of antioxidants and promising for further study.

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UDC 615.322
https://www.doi.org/10.34907/JPQAI.2021.47.11.003

DEVELOPMENT OF AN EXPRESS TECHNIQUE FOR THE ISOLATION OF INULIN FROM THE ROOTS OF THE ELECAMPANE (*INULA HELENIUM* L.)

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Inulin is a natural plant polyphructosan, the importance of which in modern medicine and pharmacy as well as the food industry is difficult to overestimate. The available patented processes for the production of inulin are characterized by a low product yield and a significant duration. The aim of the study was to develop an express technique for the isolation and quantitative determination of inulin from the roots of elecampane (Inula helenium L.). To speed up the process of extracting the biologically active substances from the elecampane roots, as well as to increase the yield of inulin, it was decided to use an ultrasonic bath. Varying the parameters of the process, it was possible to choose the optimal conditions for extracting inulin from the elecampane roots under ultrasound treatment: raw material grinding – 0.5–1.0 mm, temperature – 80°C, extraction multiplicity – 3, extraction duration – 15 min., ultrasound frequency – 35 kHz, the ratio of raw materials and extraction solvent – 1 g per 15 ml. A further increase in the extraction time under the conditions of an ultrasonic bath leads, obviously, to the destruction of water-soluble polysaccharides. The optimal conditions for the purification of the polysaccharide complex of the elecampane roots with the production of pure inulin were also selected. The proposed method makes it possible to intensify the process of obtaining inulin from the elecampane roots and reduce the duration of the process to 6–7 hours, as well as increase the product yield up to 20.63±0.36% in terms of absolutely dry raw materials. The technique can be used for express analysis of the quality of the elecampane roots and for industrial production of inulin from this type of raw material.

Keywords: inulin, water-soluble polysaccharides, ultrasound, elecampane (*Inula helenium* L.)

Inulin is a natural polypructosan, which is partially broken down in the gastrointestinal tract to fructose, the importance of which in modern medicine and pharmacy, as well as the food industry, is difficult to overestimate. The non-split part of inulin, being an active sorbent, removes a lot of toxins from the body starting from heavy metals, radionuclides up to excess low-density lipoproteins. Inulin, being a prebiotic, contributes to the normal functioning of the gastrointestinal tract, which is especially important, since, according to Roszdravnadzor, up to 90% of Russians suffer from some degree of dysbacteriosis. In addition, inulin exhibits pro-kinetic activity, stimulating the contractility of the intestinal wall and providing normal stool. On the basis of inulin, a lot of medicines and biologically active additives, including domestic ones, are produced. Due to its moisturizing and prebiotic action, inulin is used in cosmetology, in the production of creams, shower gels, shampoos and conditioners, antiperspirants, masks and serums, cosmetics for children. Inulin is a popular sweetener for diabetic patients. In addition, inulin plays the role of a fat substitute and is used for

the production of low-calorie confectionery and dairy products [1–3].

Inulin is produced only from plants by extraction with water followed by purification. The available patented processes for production of inulin are characterized by a low product yield and a significant duration, the extraction of raw materials takes up to 3–5 days [4, 5]. The main industrial sources of inulin today are specially grown raw materials: jerusalem artichoke tubers (up to 18% inulin), chicory roots (up to 40% inulin). At the same time,"chicory" inulin has contraindications for people with varicose veins and chronic respiratory diseases. Other sources of inulin are also widely known, in particular, such available plants that have significant raw materials reserves on the territory of the Russian Federation as burdock, dandelion, and elecampane [6].

Elecampane (*Ínula helenium* L. – syn.: *Aster helenium* (L.) Scop., *Aster officinalis* All., *Corvisartia helenium* (L.) Mérat, *Helenium grandiflorum* Gilib.) – perennial plant species of Elecampane (*Ínula helenium* L.) genus, sunflower family *(Asteraceae),* grows everywhere in Europe, Asia and Africa [7–9]. There is a method for production of inulin from the elecampane roots, including preparation of inulin-containing raw materials, its mechanical cleaning, washing of roots, rhizomes and stems, their crushing and mixing. The mixed and crushed pieces of raw material are extracted twice with hot water at a temperature of 75°C for 2–3 days with constant stirring. The resulting extract of inulin as a whole is treated with 96% ethyl alcohol in a ratio of 1: 1 by volume, followed by precipitation of inulin at temperature of minus 16°C. The disadvantage of this method is the duration of the process, low inulin yield and a large amount of impurities in the finished product [10].

One of the promising physical methods of influencing on substances in order to intensify technological processes is a method based on the use of mechanical vibrations in the ultrasonic range. It was found, for example, that using ultrasound with frequency of 19–44 kHz, it is possible to extract flavonoids, tannins, phenolic glycosides, coumarins, and anthocyanins from plants with the extraction process duration reduction by 1–2 orders of magnitude [11]. This not only significantly accelerates the process of extracting useful substances from plants, but also increases the yield of the main product in comparison with other extraction methods [12,13].

The purpose of the study is to develop an express technique for obtaining inulin from the elecampane roots using an ultrasonic bath.

MATERIALS AND METHODS OF STUDY

To intensify the process of extracting water-soluble polysaccharides (WSP), ultrasonic bath "Grad 40–35" was used, weighing was carried out using analytical balance "A&D GH – 202", drying to a constant mass was provided in a hot-air oven "Vityaz GP-40". Purified water was used as an extraction solvent, and the remaining process parameters were selected experimentally.

When developing the method, the elecampane roots were used, purchased in one of the pharmacies in the city of Voronezh (manufacturer – Fitofarm LLC, series 170617).

RESULTS AND DISCUSSIONS

Initially, the optimal conditions for the extraction of water-soluble polysaccharides (WSP) from the elecampane roots using an ultrasonic bath were determined. The raw material fineness, the extraction temperature, the extraction multiplicity and duration, the ratio of raw materials to extraction solvent, and the ultrasound frequency were varied. All the determinations were carried out in three repetitions. The results of the experiment are shown in Tables 1, 2, and 3.

Thus, the optimal conditions for the extraction of water-soluble polysaccharides (WSP) from the elecampane roots were selected: the raw

material fineness – 0.5–1.0 mm, the temperature is 80°C, the extraction multiplicity is 3, the extraction duration is 15 minutes, the ultrasound frequency is 35 kHz, the ratio of raw materials to extraction solvent is 1 g per 15 ml. A further increase in the extraction time under the conditions of an ultrasonic bath leads, obviously, to the destruction of water-soluble polysaccharides. The use of ultrasound with frequency above 40 kHz also leads to the destruction of biologically active substances and is not used in the production of herbal medicinal products [11].

Further studies were directed to the development of a method for the purification of the obtained water-soluble polysaccharides from the elecampane roots. The precipitate obtained after precipitation of water-soluble polysaccharides with ethanol contains impurities of pectin, some pigments, and some organic acids. To remove pectins, it was decided to provide interaction with calcium salt after dissolving the resulting WSP precipitate in water, and to purify it from pigments the interaction shall be provided with finely dispersed aluminum oxide [14]. After filtering the resulting impurity precipitate under vacuum, it was decided to remove the remaining impurities by passing the solution through the cationite and anionite columns, for which ion

Table 1

THE RESULTS OF THE QUANTITATIVE DETERMINATION OF WATER-SOLUBLE POLYSACCHARIDES (WSP) (% IN TERMS OF ABSOLUTELY DRY RAW MATERIALS) IN THE ELECAMPANE ROOTS WITH VARYING RAW MATERIAL FINENESS AND ULTRASONIC BATH TEMPERATURE (WITH THREE-TIME EXTRACTION FOR 15 MINUTES EACH USING AN ULTRASOUND FREQUENCY OF 35 KHZ, THE RATIO OF RAW MATERIALS AND EXTRACTION SOLVENT – 1 G PER 15 ML)

Temperature, °C	Raw material fineness, mm		
	0.2–0.5	0.5–1.0	1.0–2.0
60	15.32±0.40	15.19±0.34	12.25±0.50
70	22.42±0.26	24.90±0.37	18.28±0.42
80	26.12±0.25	31.57±0.44	25.99±0.39

Table 2

THE RESULTS OF THE QUANTITATIVE DETERMINATION OF WATER-SOLUBLE POLYSACCHARIDES (WSP) (% IN TERMS OF ABSOLUTELY DRY RAW MATERIALS) IN THE ELECAMPANE ROOTS WITH VARYING MULTIPLICITY AND DURATION OF EXTRACTION (WITH RAW MATERIAL FINENESS OF 0,5–1,0 MM, THE ULTRASONIC BATH TEMPERATURE OF 80°C, USING AN ULTRASOUND FREQUENCY OF 35 KHZ, THE RATIO OF RAW MATERIALS AND EXTRACTION SOLVENT – 1 G PER 15 ML)

Duration of extraction, min.	Multiplicity of extractions		
	1	2	3
10	11.34±0.40	18.47±0.40	24.68±0.34
15	15.78±0.51	20.80±0.52	31.57±0.42
20	16.80±0.32	22.97±0.60	28.96±0.40

Table 3

THE RESULTS OF THE QUANTITATIVE DETERMINATION OF WATER-SOLUBLE POLYSACCHARIDES (WSP) (% IN TERMS OF ABSOLUTELY DRY RAW MATERIALS) IN THE ELECAMPANE ROOTS WITH VARYING THE RATIO OF RAW MATERIAL TO EXTRACTION SOLVENT AND ULTRASOUND FREQUENCY (WITH THREE-TIME EXTRACTION FOR 15 MINUTES EACH, RAW MATERIAL FINENESS OF 0,5–1,0 MM, THE ULTRASONIC BATH TEMPERATURE OF 80°C)

Ratio of raw material and extraction solvent (g:ml)	Ultrasound frequency, kHz		
	15	25	35
1:10	14.68±0.30	21.86±0.43	23.49±0.30
1:15	15.86±0.45	26.37±0.30	31.57±0.44
1:20	16.90±0.37	24.30±0.52	27.96±0.46

exchange columns with anionite in the hydroxyl form AV-17-8 and cationite in the hydrogen form KU-2-8 were selected.

The degree of purification of the finished product was provided by thin-layer chromatography (TLC), comparing it with a standard inulin sample (plates – Silufol, system – 55% ethanol, developer – solutions of resorcinol and sulfuric acid diluted with subsequent heating, Rf~0.81) [15,16].

The complex of the experimental works performed makes it possible to propose the following method of isolation and subsequent quantitative gravimetric determination of inulin in the elecampane roots. To obtain inulin, the analytical sample of the raw material is crushed to particles of 0.5–1.0 mm in size. About 1 g (exact weight) of the crushed raw material is placed in a flask with a capacity of 50 ml, 15 ml of purified water heated to the boiling point is added, and placed into an ultrasonic bath with frequency of 35 kHz at temperature of 80°C, then extracted for 15 minutes. The extraction is repeated 2 more times with adding 15 ml of water. The water extracts are combined and filtered through 3 layers of gauze with a cotton swab placed in a glass funnel with a diameter of 5 cm. Precipitation is carried out with a triple amount of 95% ethyl alcohol, mixed, cooled in a freezer at a temperature of –18°C for 1 hour. The content of the flask is then filtered through a pre-dried and weighed ashless paper filter laid in a glass filter POR 16 with a diameter of 40 mm, under vacuum at residual pressure of 0.4–0.8 atm.

The resulting precipitate is dissolved in 10 ml of purified water heated to 80°C, 5 drops of a 50% solution of calcium chloride and 0.5 g of fine aluminum oxide powder are added, hold for 20 minutes, then filtered under vacuum at residual pressure of 0.4–0.8 atm. The resulting filtrate is successively passed through ion-exchange columns with anionite in the hydroxyl form AV-17–8 and cationite in the hydrogen form KU-2–8, taking into account the capacity of the ion-exchange resins to the eluate pH of 6.5–7.5 and the degree of inulin purity equal to 97%. To precipitate inulin, a three-fold amount of 95% ethanol is added to the eluate again with stirring, cooling in the freezer at –18°C for 1 hour, filtration of the precipitate is carried out through a pre-dried ashless paper filter under vacuum at residual pressure of 0.4–0.8 atm. The filtration residue is washed sequentially with 15 ml of a solution of 95% ethyl alcohol in purified water (3:1), 10 ml of mixture of ethyl acetate and 95% ethyl alcohol (1:1). The filter with the residue is dried first in air, then at temperature of 100–105°C to a constant mass.

Table 4

METROLOGICAL CHARACTERISTICS OF THE METHOD OF QUANTITATIVE DETERMINATION OF INULIN IN THE ELECAMPANE ROOTS

N	f	X	S^2	S	S_x	P, %	t(P, f)	Δx	ε, %
10	9	20.63	0.02552	0.15975	0.05052	95	2.2622	0.36	1.75

The inulin content in terms of completely dry raw materials is calculated using the standard formula:

$$X = \frac{(m_2 - m_1) \times 100 \times 100}{m \times (100 - W)},$$

where m_1 – the mass of the dried filter, g; m_2 – the mass of the dried filter with residue, g; m – the weight of the raw material, g; W – the loss in the mass of the raw material during drying, %.

The proposed method makes it possible to intensify the process of obtaining inulin from the elecampane roots and reduce the time spent on it to 6–7 hours, as well as increase the product yield to 20.63±0.36% in terms of completely dry raw materials.

The metrological characteristics are given in Table 4, where N is the number of repetitions, f is the number of degrees of freedom, X is the average value to be determined, S^2 is the dispersion, S is the standard deviation, S_x is the standard deviation of the average value, P is the confidence probability, t(P, f) is the Student t-test, Δx is the half – width of the confidence interval of the value, ε is the relative error of the average result. Thus, the relative error of the proposed method with a confidence probability of 95% is 1.75%.

CONCLUSIONS

An express technique of isolation and quantitative determination of inulin from the elecampane roots has been developed, which can be used for quality control of this type of raw material and industrial production of inulin. The optimal conditions for the extraction of water-soluble polysaccharides (WSP) from the elecampane roots were selected: the raw material fineness – 0.5–1.0 mm, the temperature – 80°C, the extraction multiplicity – 3, the extraction duration – 15 minutes, the ultrasound frequency – 35 kHz, the ratio of raw materials to extraction solvent – 1 g per 15 ml. In addition, the optimal conditions for cleaning the polysaccharide complex of the elecampane roots were selected, which are reduced to the precipitation of pectins by calcium salts, the absorption of aluminum pigments by oxide, followed by passing the extract through ion-exchange columns. The proposed technique allows you to intensify the process of obtaining inulin from the elecampane roots and reduce the process duration to 6–7 hours, as well as increase the product yield to 20.75% in terms of completely dry raw materials.

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UDC 615.322:582.998.1
https://www.doi.org/10.34907/JPQAI.2021.99.12.004

STUDY OF NITROGEN-CONTAINING COMPOUNDS OF PRICKLY LETTUCE (*LACTUCA SERRIOLA* L.)

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The object of the study was the air-dry herb of prickly lettuce (Lactuca serriola L.), harvested in 2019 in the Medvensky district of the Kursk region during the plant flowering period. The qualitative and quantitative composition of nitrogen-containing compounds of prickly lettuce herb was studied. The presence of nitrogenous bases in the herb was determined in water extracts using qualitative reactions and paper chromatography. The paper chromatography method revealed 6 compounds classified as nitrogenous bases with Rf 0.09, Rf 0.25, Rf 0.35, Rf 0.47, Rf 0.73, Rf 0.98. For the quantitative determination of nitrogenous bases, the method of G.A. Lukovnikov and A.I. Esyutina was used. This method is based on the determination of the optical density of nitrogenous bases with Reinecke salt. The content of the sum of nitrogenous bases in the prickly lettuce herb is 0.13±0.002%, including choline – 0.05±0.003%. The amino acid composition of lettuce herb is represented by 16 compounds: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine; 8 of which are essential.

Keywords: Prickly lettuce (*Lactuca serriola* L.) herb, nitrogenous bases, amino acids, chromatography, photoelectric colorimetry, high performance liquid chromatography

Prickly lettuce (*Lactuca serriola* L.) of the sunflower family *(Asteraceae)* has long been used in folk medicine in Russia as an antipyretic, anti-inflammatory, analgesic, antitumor, blood purifying agent [1,2]. When taking prickly lettuce, appetite improves, headaches and cough decrease; it can treat jaundice, leprosy, insomnia. Boiled lettuce is used to increase the secretion of milk and to treat the chest organs. Lettuce leaves are used in case of ligament tension in the form of bandages [2]. The prickly lettuce has attracted the attention of scientists since the discovery of milky juice in it, which is used for the same purpose as lettuce herb [2]. Experimental studies of extracts obtained by various extraction solvents from the aboveground part of lettuce have established the antioxidant activity by the reaction of free radicals with 1,1-diphenyl-2-picrylhydrazyl. The most pronounced activity was observed in the ethyl acetate fraction. The antioxidant effect was also found in flavonoids and sesquiterpene 11/3, 13-dihydrolactucin isolated from lettuce herb [3]. Methanol extract from the herb showed antispasmodic, analgesic, vasodilating, antitumor, and bronchodilatory effects [4–6]. Triterpene compounds isolated from the aboveground part of the plant exhibit anti-inflammatory, antibacterial, antitumor, cytostatic, and antimalarial

effects [5]. In the literature, there is evidence that lettuce exhibits an antidiabetic property, reducing blood sugar levels [2].

Prickly lettuce is represented by annual or biennial herbaceous plants growing on the territory of the European part of Russia, in the Far East, in Western and Eastern Siberia, in the Caucasus [1]. In the regions of central Russia, it grows on waste lands, weed-grown places, roadsides, in vegetable gardens, orchards [1]. It naturally forms significant thickets and has a sufficient raw material base. However, to use lettuce as a raw material source, data on its chemical composition is necessary. In the literature, information about the composition of biologically active substances of lettuce is rare, and the studies were conducted mainly by foreign scientists. The most common information is about the presence of sesquiterpene lactones, which are found in all the organs of the plant, in the aboveground part, the steroid and triterpene compounds are also established; flavonoids are also identified in it. The leaves additionally contain a complex of vitamins [6]. The seeds were tested for the content of alkaloids, sesquiterpenoids, triterpenoids, essential and fatty oils [2,6].

Purpose of this work is study of nitrogen-containing compounds in the prickly lettuce herb.

MATERIALS AND METHODS

The aboveground part of the prickly lettuce collected in the Medvensky district of the Kursk region in 2019 during the flowering phase was selected as the material for the study. The collected raw materials were dried in the air in a shaded place. For the analysis of nitrogen-containing compounds, an average sample was used, from which an analytical sample was taken and crushed to a particle size of 1 mm. The determination of nitrogen-containing compounds included the determination of nitrogenous bases and amino acids.

For the qualitative analysis of nitrogenous bases and amino acids from the analytical sample of raw materials (5.0 g), an aqueous extract was obtained by three-time extraction with purified water of 50 ml, each extraction was carried out for 1 hour. The resulting aqueous extracts were combined, evaporated to a volume of 25 ml under vacuum, and used for qualitative analysis of nitrogenous bases and amino acids. For the qualitative analysis of nitrogenous bases, qualitative reactions were used: with Mandelin reagent, with phosphoric-tungsten acid, with a 3% diamond green solution, and with hydrochloric acid; as well as the method of paper chromatography was used, the solvent system in which was the system "n. butanol – acetic acid – water" (4:1:2), the developer was iodine vapor [7].

Qualitative analysis of amino acids was performed using the ninhydrin reaction and the thin-layer chromatography method [8]. Chromatographic analysis was performed on Sorbfil plates using the solvent system "96% ethyl alcohol – concentrated ammonia" (16:4,5) and reliable samples of amino acids. The developer of the chromatogram was a 0.2% ninhydrin solution, after which the chromatogram was held in a drying cabinet at 100–105°C for a few minutes. At the same time, red-purple spots of amino acids appeared.

The quantitative analysis of nitrogenous bases was carried out by the photoelectrocolorimetric method, using the modified method of G.A. Lukovnikov and A.I. Esyutina. The method is based on the formation of colored complexes of nitrogenous bases contained in raw materials with Reineke salt, and the determination of their optical density. The extract was obtained with purified water and used to determine the choline and the amount of nitrogenous bases. To determine choline, hydrochloric acid was added to the resulting extract to provide pH=3 and a solution of Reineke salt, then placed in the refrigerator for 18 hours to obtain a precipitate of nitrogenous

bases. The precipitate of the resulting complex was separated by filtration, dissolved in acetone, and colorimetrated at a wavelength of 400±10 nm (blue light filter) on a photoelectrocolorimeter. Under the same conditions, a solution of a standard sample of choline with Reinecke salt was colorimetrated [7]. The amount of nitrogenous bases was determined in the same water extract, after adding a potassium permanganate 0.1 N solution to it and heating it in a water bath for 10 minutes to oxidize the nitrogenous bases to choline. Further determination was carried out according to the method of determining choline [7].

The amino acid composition was studied by high-performance liquid chromatography using an Agilent 1260 device with a fluorescent detector (FLD), for which aqueous extract was used. When determining the content of free amino acids, the aliquot of aqueous extract and standard solutions (in the form of aqueous solutions) of amino acids were derivatized with the reagent ACCQ FLUOR, mixed using a vortex mixer and incubated for 10 minutes at temperature of 55°C. The resulting solutions were introduced into a chromatographic column and analyzed using the following conditions: gradient elution method, mobile phase flow rate – 1 ml/min, excitation wavelength – 250 nm, emission – 395 nm [9].

RESULTS AND DISCUSSION

Positive results of qualitative reactions to the presence of nitrogenous bases indicate their presence in the prickly lettuce herb. By the method of thin-layer chromatography, 6 substances were found: with Rf 0.09, Rf 0.25, Rf 0.35, Rf 0.47, Rf 0.73, Rf 0.98, classified as nitrogenous bases. Photoelectrocolorimetric determination of nitrogenous bases showed that their sum is 0.13±0.002%, including choline 0.05±0.003%.

When conducting a qualitative analysis of amino acids, the formation of red-purple color

Table

AMINO ACID COMPOSITION

Name of amino acids	Content of amino acids	
	mg/100 g in raw material	% sum
Aliphatic amino acids *Monoaminomonocarboxylic*		
Alanine	4.89	10.53
Valine*	6.40	4.02
Leucine*	19.34	21.36
Isoleucine*	0.73	0.63
Glycine	12.24	9.39
Total content	43.61	45.93
Monoaminodicarboxylic		
Glutamine acid	9.29	3.55
Asparaginic acid	4.30	2.42
Total content	13.59	5.97
Diaminomonocarboxylic		
Arginine	20.52	16.95
Lysine*	1.63	3.08
Total content	22.15	20.03
Oxymonoaminocarboxylic		
Threonine *	3.60	1.94
Serine	10.79	4.55
Total content	14.39	6.49
Sulfur-containing substances		
Methionine*	0.34	7.50
Total content	0.34	7.50
Aromatic amino acids		
Tyrosine	3.16	0.60
Phenylalanine*	4.90	6.05
Total content	8.06	6.65
Heterocyclic amino acids		
Histidine*	2.64	4.36
Proline	14.79	3.07
Total content	17.43	7.43
Content of essential amino acids	39.58	

*Note: * – essential amino acids*

staining with ninhydrin reagent indicates their presence in the prickly lettuce herb. Chromatographic analysis of the amino acid composition with a certain degree of confidence showed the presence of arginine, alanine, leucine, methionine, and glycine.

The results of the study of the amino acid composition by high-performance liquid chromatography revealed the presence of 16 amino acids, including 8 essential ones (see Table.)

The amino acid profile of prickly lettuce herb includes aliphatic (monoaminocarboxylic, monoaminodicarboxylic, diaminomonocarboxylic, oxymonoaminocarboxylic, sulfur-containing), aromatic, and heterocyclic amino acids. Among them, aliphatic amino acids predominat; their content is 85.92% in the total of amino acids.

Among the aliphatic amino acids, monoaminocarboxylic acids predominate, the content of which is 45.93% in the total of acids, followed by diaminocarboxylic acids (20.03%). The lowest content among them was found in monoaminodicarboxylic acids (5.97% in total). The content of aromatic (6.65%) and heterocyclic (7.43%) is approximately at the same level. The content of essential amino acids was 39.58 mg/100 g. Thus, the prickly lettuce herb can be considered as an additional source of amino acids.

CONCLUSIONS

1. The qualitative composition and quantitative content of nitrogen-containing compounds (nitrogenous bases, amino acids) of prickly lettuce (*Lactuca serriola* L.) herb have been established. The content of the sum of nitrogenous bases in the prickly lettuce herb is 0.13±0.002%, including choline 0.05±0.003%.

2. The qualitative and quantitative amino acid composition of the prickly lettuce herb has been established: amino acids are represented by 16 compounds, of which 8 are essential. Among the identified groups of amino acids, the maximum content was found in monoaminodicarboxylic amino acids (45.93%).

3. The conducted studies allowed us to expand the composition of biologically active substances of prickly lettuce herb, which can contribute to their further use in medical practice as sources of these groups of natural compounds, as well as contribute to their further study for creation of drugs based on them.

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UDC 615.322
https://www.doi.org/10.34907/JPQAI.2021.21.12.005

DEVELOPMENT OF METHODS FOR STANDARDIZATION OF PRICKLY THISTLE (*CIRSIUM ARVENSE* L.) HERB ACCORDING TO THE CONTENT OF FLAVONOIDS

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The article presents the results of the development of the method of standardization of the prickly thistle (Cirsium arvense L.) herb according to the content of flavonoids. The conditions for the quantitative determination of plant flavonoids were studied and the optimal extraction parameters were selected, such as the concentration of the extraction solvent, the ratio of raw materials to extraction solvent, fineness factor, the time and frequency of extraction, the concentration and amount of the complexing additive added. It is established that the best extraction solvent for the prickly thistle (Cirsium arvense L.) herb is ethyl alcohol at a concentration of 40%, the ratio of raw materials and extraction solvent is 1: 30, the fineness factor is 2 mm, the optimal extraction time is 30 minutes with triple extraction. It is established that the dominant substance is apigenin, which is proposed to be recalculated, and the analytical wavelength for the quantitative determination of flavonoids is 388±2 nm.

Keywords: Prickly thistle (*Cirsium arvense* L.), herb, flavonoids, apigenin, quantitative determination

Medicinal herbs are increasingly attracting the attention of researchers and practical medicine, as they have a wide range of pharmacological activity and can be effectively used for the prevention and treatment of various diseases. The possibility of a rational combination of medicinal plants both among themselves and with chemical medicinal products to enhance the therapeutic effect, their softness of action, the rare manifestation of side effects are the main advantages of medicinal plants. In this regard, a promising medicinal plant for studying is the Prickly thistle (*Cirsium arvense* L.) of the sunflower family *(Asteraceae),* which is widely used in folk medicine as an anti-inflammatory, antioxidant and antimicrobial agent, it is used as a remedy for gout and rheumatism, externally for skin diseases, used for various nervous diseases, epilepsy, diseases of the digestive system [2,3]

Purpose of study is development of methods for standardization of Prickly thistle (*Cirsium arvense* L.) herb according to the content of flavonoids.

MATERIALS AND METHODS

As the objects of the study, we used samples of Prickly thistle (*Cirsium arvense* L.) herb, harvested on the territory of the Republic of Bashkortostan.

The quantitative determination of flavonoids was carried out by the method of differential spectrophotometry in the ultraviolet region using a Shumadzu UV-1800 spectrophotometer with the selection of optimal extraction parameters: extraction solvent, the ratio of raw materials to extraction solvent, fineness factor, the time and frequency of extraction, the conditions for the complex formation reaction. Statistical data processing was carried out in accordance with the requirements of the State Pharmacopoeia of the Russian Federation [1].

RESULTS AND DISCUSSION

The method of differential spectrophotometry with the selection of optimal conditions was used to develop a technique for the quantitative determination of flavonoids in the Prickly thistle herb [4,5]. In the course of the experiment, the absorption spectra of alcohol solutions of the Prickly thistle herb, as well as solutions with adding a complexing additive – a solution of aluminum chloride (III), were studied to exclude the influence of excipients. With a solution of aluminum chloride, flavonoids form complexes that

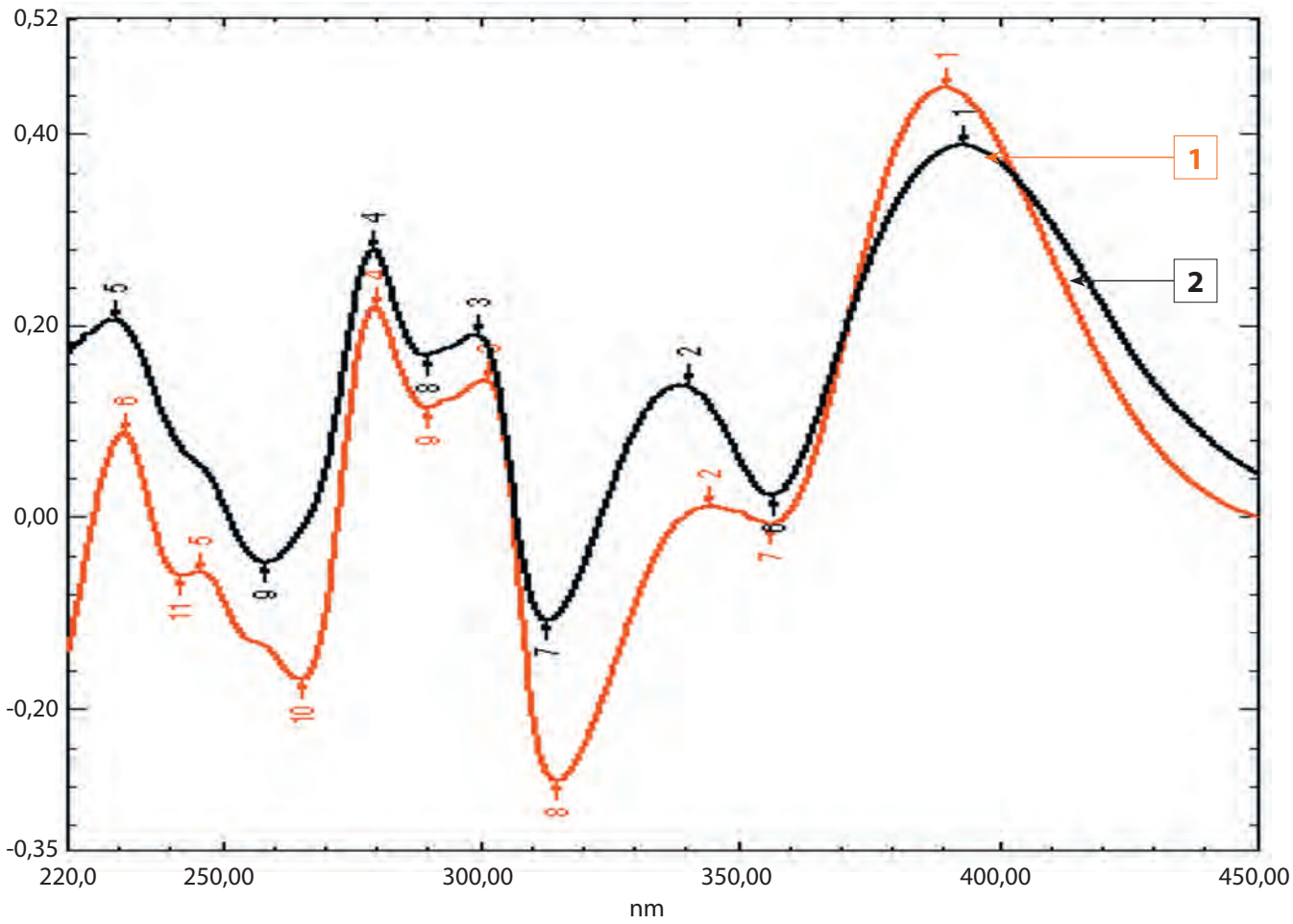

FIG. 1. *Differential spectra:*
1 – SUBSTANCES *of the standard – apigenin; 2 – extracts from Prickly thistle herb with the addition of an aluminum chloride solution (λ_{max}=388±2 nm)*

are stable in acidic environment, and a bathochromic shift is observed with clearly defined absorption maxima (Fig. 1).

It was found that the absorption spectrum of the Prickly thistle herb has a close maximum to the spectrum of apigenin (λ_{max}=388±2 nm), so this flavonoid was chosen as the main one in the amount for which it is proposed to recalculate in the future. The resulted data were consistent with the results of the previously conducted chromatographic analysis of alcohol extracts from the Prickly thistle raw materials.

At the next stage, it was necessary to select the conditions for the extraction of flavonoids that would ensure their maximum yield from the Prickly thistle raw material, namely: concentration of the extraction solvent, the raw material fineness (since the particle size of the raw material affects the completeness and speed of transition to the solution of the substances under study), the ratio of the raw material and the extraction solvent, the time and frequency of extraction.

In a comparative assessment of the effect of the extraction solvent on the yield of flavonoids, ethyl alcohol of various concentrations was used. In the resulted extracts, the optical density was determined in the range of wavelengths which are characteristic of flavonoids, and the content of flavonoids was calculated (Table 1). As a result, it was found that the optimal extraction solvent, when using which the higher values of the content of flavonoids were observed, is 40%

Table 1

EFFECT OF THE EXTRACTION SOLVENTS ON THE YIELD OF FLAVONOIDS

Concentration of ethyl alcohol	Optical density (D average)	Content of flavonoids, %
40%	0.528±0.025	2.89±0.14
50%	0.427±0.022	2.73±0.12
70%	0.425±0.019	2.68±0.11
80%	0.376±0.017	2.51±0.09
90%	0.364±0.008	2.46±0.07

Table 2

EFFECT OF EXTRACTION PARAMETERS ON FLAVONOID YIELD

Parameter	Content of flavonoids, %	
Fineness of raw materials, mm		
1	2.77±0.13	
2	2.84±0.18	
3	2.69±0.10	
Extraction time, min		
15	2.64±0.07	
30	2.88±0.12	
45	2.75±0.09	
60	2.59±0.07	
90	2.39±0.04	
Ratio of raw material and extraction solvent		
1:30	2.84±0.12	
1:50	2.77±0.10	
1:100	2.68±0.08	
Frequency of extraction		
1:30	one-time	2.72±0.03
	two-fold	2.88±0.05
	three-fold	2.92±0.06
1:50	one-time	2.64±0.04
	two-fold	2.75±0.07
	three-fold	2.79±0.11
1:100	one-time	2.59±0.04
	two-fold	2.62±0.05
	three-fold	2.68±0.02

ethyl alcohol, which was selected for further studies.

The results of studies for selection of the fineness factor of raw materials, the extraction time, the ratio of raw materials and extraction solvent, the frequency of extraction are presented in Table 2.

According to the resulting data, the optimum way is the fineness of raw materials to the size of particles passing through a sieve with a mesh diameter of 2 mm, the extraction time at which the maximum extraction of flavonoids occurs is 30 minutes, the ratio of raw materials to extraction solvent is 1: 30 and three-time extraction provides the most complete extraction of flavonoids from the Prickly thistle raw materials.

To determine the optimal parameters of the effect of complexing agent on the concentration of flavonoids, the concentration of an alcoholic solution of aluminum chloride and its amount added to the extraction were studied (Table 3).

Analyzing the data obtained, it can be noted that the most optimal way is the use of a 2% solution of aluminum chloride in amount of 1 ml. When assessing the stability of the resulting complex, it was found that the complex formation reaction develops within 45 minutes and the complex remains stable for an hour.

On the basis of the conducted studies, a method for the quantitative determination of flavonoids in the Prickly thistle herb is proposed: an analytical sample of raw materials is crushed to the size of particles passing through a sieve with a mesh diameter of 2 mm. About 1 g (exact weight) of the raw material is placed into a flask made of heat-resistant glass with a slice with a capacity of 250 ml, 30 ml of 40% ethyl alcohol is added, then it is attached to the backflow condenser and heated in a boiling water bath for 30 minutes. Then the resulting extraction is carefully filtered through cotton wool into a 100 ml volumetric flask, avoiding the ingress of raw material particles into the funnel. To the remaining raw materials in the flask, 30 ml of 40% ethyl alcohol is added and cotton wool, through which the solution was filtered, is attached to the backflow condenser and heated in a boiling water bath for another 30 minutes. The content of the flask is filtered into a measuring flask with the first portion of extraction through cotton wool. The process is repeated one more time according to the above procedure. The resulting extraction is brought to 100 ml in a measuring flask with the same solvent (solution A)

Into a measuring flask with a capacity of 25 ml, 1 ml of solution A is placed, 1 ml of a 2% alcohol solution of aluminum chloride is added

Table 3

EFFECT OF THE COMPLEXING AGENT ON THE YIELD OF FLAVONOIDS

Study sample	Content of flavonoids, %				
	Aluminum chloride concentration				
	1%	2%	3%	5%	10%
Thistle herb	2.63±0.09	2.78±0.12	2.66±0.10	2.54±0.08	2.48±0.09
	Amount of aluminum chloride added				
	1 ml	2 ml	3 ml	5 ml	10 ml
	2.80±0.11	2.68±0.09	2.54±0.08	2.51±0.06	2.44±0.05

Table 4

THE METROLOGICAL CHARACTERISTICS OF THE METHOD OF QUANTITATIVE DETERMINATION OF FLAVONOIDS IN THE PRICKLY THISTLE HERB

Object under study	f	$\bar{x}$	s^2	S_x	P, %	t(P, f)	E_α	ε,%
Prickly thistle herb	5	2.78	0.000038	0.0019	95	2.57	0.09	3.23

and the solution is brought to the mark with 95% ethyl alcohol (solution B). After 45 minutes, the optical density of the solution is measured using a spectrophotometer at a wavelength of 389 nm in a cuvette with a layer thickness of 1 cm. As a reference solution, a solution consisting of 1 ml of A solution and 1 drop of a solution of diluted acetic acid, brought to the mark with 95% ethyl alcohol is used in a measuring flask with a capacity of 25 ml.

In parallel, the optical density of the complex of a solution of an apigenin standard sample with a solution of aluminum chloride is measured: 1 ml of a 2% alcohol solution of aluminum chloride is added to 1 ml of a 0.05% solution of apigenin and the solution is brought to 25 ml with 95% ethyl alcohol in a measuring flask. The measurements are carried out similarly to the test solution

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

$$X = \frac{A \times a_0 \times 100 \times 1 \times 25 \times 100 \times 100}{A_0 \times a \times 25 \times 1 \times (100 - W)},$$

where A is the optical density of the analyzed solution (solution B); A0 is the optical density of the complex of the standard sample of apigenin with aluminum chloride; a is the weight of the raw material in grams; a0 is the weight of the standard sample of apigenin in grams; W is the weight loss of the raw material during drying, %.

The metrological characteristics of the method of quantitative determination of flavonoids in the Prickly thistle herb are presented in Table 4, the error of the experiment was not higher than the maximum permissible values.

CONCLUSIONS

1. A method has been developed for the quantitative determination of the amount of flavonoids in the prickly thistle herb by differential spectrophotometry in terms of apigenin (λ_{max}=388±2 nm)

2. The optimal parameters of extraction of flavonoids in the prickly thistle raw materials were established: the extraction solvent – ethyl alcohol at concentration of 40%, the ratio of raw materials and extraction solvent – 1:30, the fineness factor – 2 mm, the extraction time – 30 minutes with three-fold extraction, the concentration of the complexing agent – 2%, the amount of the added complexing agent – 1 ml

3. The content of the sum of flavonoids in terms of apigenin is 2,78±0,09%.

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UDC 615.453.8
https://www.doi.org/10.34907/JPQAI.2021.52.69.006

STUDY OF THE PHYSICOCHEMICAL AND TECHNOLOGICAL PROPERTIES OF THE BASE FOR HEALTH IN GUM® MEDICAL CHEWING GUM

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Medicinal chewing gum is a dosage form that is gaining popularity due to the ease of use (does not require water), the gradual release of medicines in the oral cavity, reducing the risk of side effects due to the lack of direct contact with the gastric mucosa and other benefits. This study demonstrated the physicochemical and technological properties of the base for the preparation of Health in Gum® PWD-04 chewing gum (gum bases) by direct compression. The analysis of the resulting data is carried out and the conclusion is made about the applicability of the direct compression process for the studied brand of gum base.

Keywords: medicinal chewing gum, gum base, base for medicinal chewing gum, Health in Gum®, technological properties, particle shape and size

The oral route of administration of medicines is the most preferred among patients and physicians due to some advantages over other dosage forms (DF). One of the reasons is the ease of administration and the possibility of use for people suffering from dysphagia, in addition, some active substance (AS) are absorbed in the oral cavity [1–3]. Medicines with significant buccal absorption, such as oro-dispersible tablets, chewing tablets, and chewing gum, provide a rapid therapeutic effect compared to DF for oral administration.

Chewing tablets and chewing gums are especially actively used in pediatrics as more preferred dosage forms compared to oral liquids and tablets. In addition, chewing gums are kept in the oral cavity for a long time, which provides local use for some medicines. According to SP XIV, "medicinal chewing gum is a solid dosage form of "rubber-like" consistency intended for chewing for a certain period of time without subsequent ingestion in order to provide local action in the oral cavity and throat or systemic action." Therefore, due to the presented features and ease of use, a medicinal chewing gum is a promising dosage form for oral use.

The purpose of this work is to describe the excipients used in the production process for medicinal chewing gum, as well as to study the physical, chemical and technological properties of one of the brands of bases for medicinal chewing gum (gum bases).

MATERIALS AND METHODS

During the research, the physicochemical and technological properties of the base for Health in Gum® PWD-04 medicinal chewing gum (Cafosa®, Spain) were studied according to the following characteristics.

Assessment of the flow character and measurement of the angle of repose (OFS.1.4.2.0016.15 SP XIV) was performed using an Erweka GDT vibrating funnel. Optical microscopy (OFS.1.1.0015.15, SP XIV) was carried out using a microscope (Nikon, Eclipse E200). Weight loss during drying (OFS.1.2.1.0010.15, KV XIV) was measured using a Sartorius MA-35 moisture meter. Compressibility was checked using a PRG-50 manual hydraulic press. Tablet compression test (OFS.1.4.2.0011.15, SP XIV) was performed using a TBF 1000 mechanical strength tester (Copley Scientific®). The bulk weight (bulk density) was measured using an Erweka SVM 221 instrument. The Carr's index was calculated using the formula:

$$Ic = [(\rho_2 - \rho_1) / \rho_2] \times 100\%;$$

the Hausner coefficient was calculated using the formula:

$$k_H = \rho_2 / \rho_1,$$

where $\rho_1 = M/V_1$ is the bulk density, and $\rho_2 = M/V_2$ is the bulk density after compaction. The true density was determined using a pycnometer for powdered substances (volumeter) filled with liquid, according to the formula:

$$\rho = \frac{m \times \rho_l}{m + m_1 + m_2},$$

where m is the mass of the substance, g; ρ_l is the density of the liquid, g/cm^3; m_1 is the mass of the volumeter with the substance, g; m_2 is the mass of the volumeter with the liquid and substance, g. The porosity was calculated based on the density and bulk density values:

$$П = (1 - P/\rho) \times 100\%,$$

where П is the porosity, %; P is the bulk density, g/cm^3; ρ is the density, g/cm^3.

RESULTS AND DISCUSSION

Chewing gum is a mixture of natural or synthetic resins and resins sweetened with sugar, corn syrup, artificial sweeteners, and may also contain colorants and flavors.

Chewing gum consists of two parts:

1. Water-insoluble chewing gum base.
2. Water-soluble constituent.

1. The water-insoluble base of chewing gum usually contains polymers (elastomers), resins, fats, oils, and inorganic fillers [4].

a) Elastomers are polymer compounds with high elasticity properties. The elastomer provides flexibility to tear and break and controls the regular texture.

There are natural and synthetic types of elastomers, the most commonly used are natural resins, such as: Jelutong, LechiCaspi, Perillo, Chicle.

b) Plasticizers are necessary to obtain a variety of desired textures and consistency properties, reduce brittleness, and facilitate the mixing of other components. Excipients such as glycerin, lanolin, palmitic acid, oleic acid, stearic acid, potassium stearate, microcrystalline waxes, propylene glycol, terpene resins derived from α-pinene and/or d-limonene are used.

c) Fillers or texturizers form the texture, improve the chewable properties, provide a reasonable size of a chewing gum with a low dose of a pharmaceutical substance. Commonly used fillers are magnesium and calcium carbonate, ground limestone, magnesium and aluminum silicate, aluminum hydroxide, talc, titanium oxide, and calcium mono -, di -, and triphosphate.

2. Water-soluble excipients consist of sweeteners, flavors, emulsifiers, dyes and antioxidants [5].

In addition, the composition for compression includes excipients, improving the flowability and anti-adhesive properties of the mixture for tableting (lubricants and glidants): silicon dioxide, magnesium stearate, talc can be used in medicinal chewing gum.

In addition to the independent selection of these components, there is an alternative direction in the production of medicinal chewing gum, which consists in the use of a ready-made base for chewing gum, which contains the main components of the formulation. An example of such a base is Health in Gum® (Cafosa®), which was used to study physical, chemical and technological properties.

This combined mixture of excipients is an inert and insoluble product used as a base for chewing gum [6]. Health in Gum® belongs to the group of free-flowing resin-like substances for direct compression developed by Cafosa Gum SAU. The presented bases for production of medicinal chewing gum contain a high percentage of inert soft thermoplastic elastomers, a mixture of polyols (sorbitol/xylitol/mannitol), sugars, plasticizers and anti-adhesives. When compressing the Health in Gum®, chewing gums are produced, which are similar in appearance to tablets for oral use. They have a higher hardness and friability than medicinal chewing gum, made by the traditional method of extrusion. Health in Gym® has three varieties: HiG PWD-01, HiG PWD-03 и HiG PWD-04, which contain 25, 35 and 30% of elastomeric base, respectively.

The results of studies of the physico-chemical and technological parameters of the HiG PWD04 base are presented in Table. 1 and in Figures 1 and 2.

Health in Gum® is a mixture of anisometric particles with different shapes or (Fig. 1) in the form of conglomerates of particles with the sizes of the main fractions from 500 to 1000 μm (<50%) and 100–300 μm (<70%) (Fig. 2). Based

Table 1

PHYSICO-CHEMICAL AND TECHNOLOGICAL PARAMETERS OF THE BASE FOR HEALTH IN GUM® MEDICINAL CHEWING GUM

Parameter	Units	Values
Description	White with a yellowish tint, inhomogeneous amorphous powder	
Flowability	g/s	4.02±1.05
Compressibility (fractural strength)	N	4.56±0.17
Angle of repose	Degrees	40±13
Residual moisture	%	1.03±0.60
Bulk weight before compression	g/cm^3	0.608±0.012
Bulk weight after compression	g/cm^3	0.621±0.021
Carr's index	%	2.13±0.01
Hausner coefficient		1.02±0.02
Porosity	%	54.302±3.503
True density	g/cm^3	1.3304±0.0032

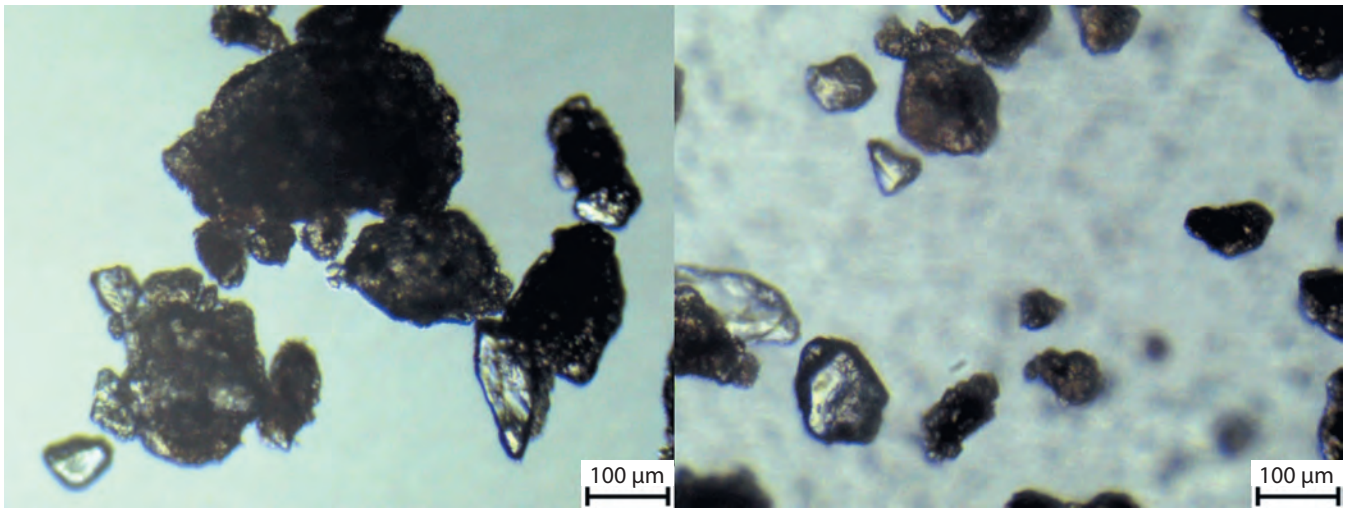

FIG. 1. *Micrographs of the particles of the base for Health in Gum® medicinal chewing gum (magnification 160×)*

on the results of optical microscopy, it can be concluded that the particles of the gum base are heterogeneous in shape and size. There are several main fractions and the largest particles are translucent equilateral agglomerates with pitted surface and inclusions. The remaining fractions are described as translucent angular rough equilateral or transparent angular smooth plate-like particles.

Despite the relatively large distribution of particles in size and shape, gum base has a very good (excellent) degree of flowability, which is also specified by the Hausner coefficient, whose values do not exceed 1.26.

Due to the presence of elastomers in the base for medicinal chewing gums, through the compression a tablet with high elastic-plastic properties is produced, which is indirectly confirmed by a low Carr's coefficient and a low compressibility value. In addition, the tablets are characterized by a low ejection force (<1 kN), but sometimes the base may stick to the punches due to the heating of part of the elastomers. Gum base is also characterized by a moderate degree of porosity and low moisture content. Based on the resulting characteristics, we have identified the applicability of the Health in Gum® base in direct compression technology.

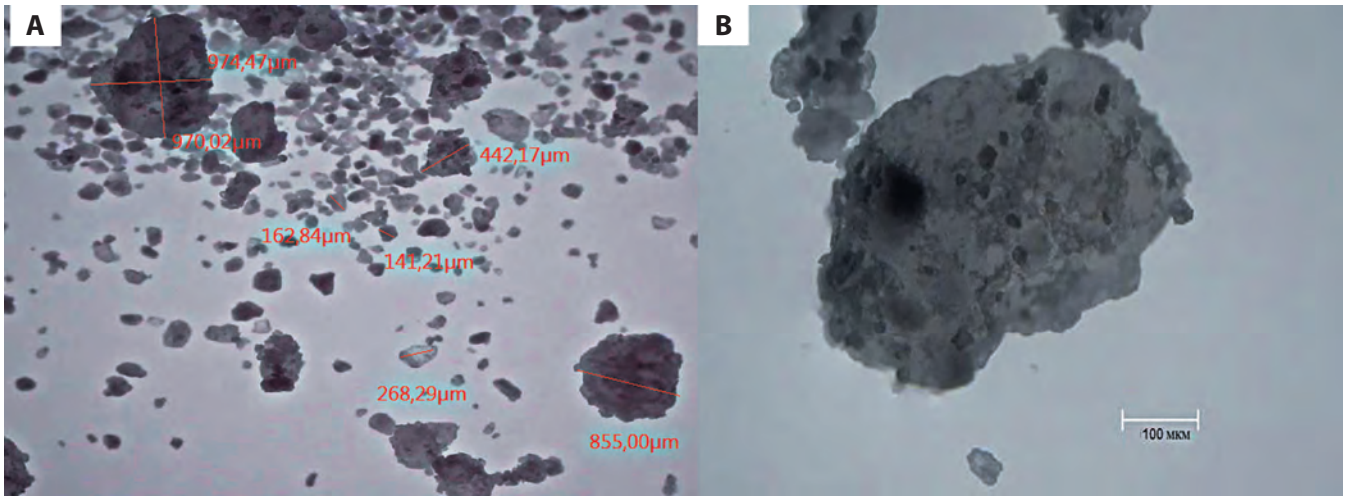

FIG. 2. *Micrographs of the particles of the base for Health in Gum® medicinal chewing gum: A) magnification 30×; B) magnification 195×*

CONCLUSIONS

As a result of the studies, it can be concluded that the base for Health in Gum® medicinal chewing gums has optimal physical, chemical and technological properties, which characterize it as a highly free-flowing powder with a wide particle size distribution, a low degree of compressibility, moderate porosity, low moisture content, and elastic-plastic properties when tableting. Based on its characteristics, this gum base can be used in direct compression technology.

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UDC 615.014.4
https://www.doi.org/10.34907/JPQAI.2021.81.36.007

THE ASPECTS OF PHARMACEUTICAL DEVELOPMENT OF THE TABLETS BASED ON DRY EXTRACTS

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Determination of the critical parameters of the process is one of the important aspects of the pharmaceutical development of medicines of consistently high quality. The purpose of this work is to identify factors that can potentially affect the quality and stability of a tableted medicine containing althea dry extract – "Mukaltin, 50 mg tablets". To determine the parameters that are critical in the production of mukaltin tablets, the influence of a number of factors on the stability of this medicine during storage was studied: the humidity of the initial components; the residual moisture of the finished tablet; the physical and chemical properties of the auxiliary component (isomerization of tartaric acid); the material of the primary packaging. In the course of the study, it was found that among the factors studied, the critical parameters of the production process of mukaltin tablets include the humidity of the intermediates and the finished product and the packaging material.

Keywords: dry extract, tablets, Mucaltin, stability, storage

Herbal medicinal products, including tableted medicines based on plant extracts, are widely used in the pharmaceutical industry and have been used for a long time due to their high efficiency, availability, ease of use and low toxicity [1,8].

In addition to the obvious pharmacological advantages, this dosage form has certain technological features. Dry extracts for the most part have high hygroscopicity, as a result of which the pharmaceutical development of a tablet dosage form based on them requires a special approach [5,7,9].

One of the important aspects of the pharmaceutical development of consistently high quality medicines is the determination of critical parameters of the process [10].

The purpose of this work is to identify factors that can potentially affect the quality and stability of a tableted medicine containing althea dry extract – "Mukaltin, 50 mg tablets" (hereinafter referred to as mukaltin tablets) [2,6].

To determine the parameters that are critical in the production of mukaltin tablets, the influence of a number of the following factors on the stability of this medicine during storage was studied:

- moisture of the initial components;
- residual moisture of the finished tablet;
- the physical and chemical properties of the auxiliary component (isomerization of tartaric acid);
- packing material.

In order to determine as accurately as possible the factor or factors that have the most pronounced effect on the stability of the medicine "Mukaltin, 50 mg tablets", as well as in order to distinguish factors from each other (since a set of factors may participate in this process), the following assumptions were made:

1. Residual moisture of the tablet. If we assume that the packaging insulates the tablets as much as possible from the effects of external moisture, it is possible that the residual moisture of the tablets may be sufficient for the occurrence and course of a gas formation reaction [4,5].

2. Influence of the packaging material on the stability of the tablets. The permeability of the packaging material may well provoke a gas formation reaction in the tablet, taking into consideration that most of the components are hygroscopic substances [11].

3. Influence of physical and chemical properties of excipients (isomerization of tartaric acid). The tablets of mukaltin as an excipient include tartaric acid. It has isomers that have different properties [5,6].

MATERIALS AND METHODS

The following components were used for the production of mukaltin tablets [2,6]:

- althea dry extract ("Harms", Russia);
- tartaric acid – D-isomer (Rono Chem Co. Ltd, China);
- tartaric acid – D–L-isomer (KOHO, China);
- sodium hydrocarbonate (Bashkir Soda Company JSC, Russia);
- calcium stearate (Khimresurs LLC, Russia).

As a reference substance, mukaltin tablets were taken, produced by Obnovlenie JSC, Russia.

Packing material:

- polyvinylchlorid (PVC) with thickness of 200 μm;
- polyvinyldichloride (PVDC) with thickness of 200 μm.

The following methods were used to determine the quality parameters [3]:

- description of tablets in the package during storage:

a) the appearance of the tablets – the color was determined by the intensity of darkening in comparison with a tablet stored at constant humidity of 10±5% and temperature of 30°C (designation on a scale of 0), and a tablet stored at constant humidity of 75±5% and temperature of 30°C (designation on a scale of 3);

b) the appearance of the primary package is the presence or absence of cell swelling (where 0 is the absence of swelling, and 4 is the state in which a slight compression of the package cell causes it to break) [4].

The sum of values characterizes the overall assessment of two parameters in four storage conditions:

- weight loss during drying;
- change in tablet weight during storage;
- resistance to crushing of tablets.

Equipment:

- climatized chamber KBF 1020 (Binder) at operation conditions 30°C and humidity 75±5%;
- hot air oven TC-80M-2 at operation conditions 40°C;
- tablet strength tester TBH 125 (Erweka);
- scales BM-2202 (Vesta Experimental Design Bureau);
- Weight Humidity Analyzer АВГ-60 (Gosmetr).

RESULTS AND DISCUSSION

Immediately before the production of the tablets and during the process, the mass loss during drying was determined as components separately (Table 1), and the tablet mixture (Table 2) [10].

Further, for the experiment, 8 types of experimental samples of mukaltin tablets and 1 type of packaging with a reference substance repacked in a PVC blister were produced and packed (Table 3).

Table 1

WEIGHT LOSS DURING DRYING OF THE COMPONENTS OF MUCALTIN TABLETS

Climatic conditions in the laboratory room: t=22,7°C, humidity – 20,6%		
Component	**Drying temperature**	**Weight loss during drying, %**
Tartaric acid D, solid	100°C	0.77
Tartaric acid D, crushed		0.78
Tartaric acid (D,L)		0.56
Sodium hydrocarbonate		1.22
Althea dry extract		2.05
Calcium stearate		1.06

All tablets after packaging had the following parameters:

- Weight of a tablet: 0,300±0,005 g;
- Tablet fractural strength: 110±10 N;
- Height of a tablet: 3,82±0,1 mm;
- Weight loss when drying tablets that have not passed the drying stage: 1,75±0,1%;
- Weight loss during drying of tablets that have passed the drying stage: 0,94±0,1%.

The tablets were stored under four different conditions [7]:

1) at 25°C and humidity 40±5% ("at room temperature" is designated as "at r.t.", Table 4);

2) at 40°C and humidity 40±5% ("in thermostat" is designated as "therm", Table 5);

3) at 30°C and humidity 75±5% ("in a climatic chamber simulating a zone 4B", is designated as "cc", Table 4);

4) in a double dense PEG bag at 25°C and humidity of 40±5% ("to exclude external influences of moisture" is designated as "package", Table 4).

Each week, the appearance was inspected visually, and each month, some of the blisters were selected for testing.

The results of inspections (after 3 months) are shown in Table. 4 and 5.

After 3 months of testing, all the samples in the climatic chamber changed their appearance: the packaging blisters were swollen, the tablets were very dark. The average mass of the samples also decreased due to the gas formation reaction (the mass loss was about 20%). None of the studied samples showed stability in the conditions of the climatic chamber.

In a double plastic bag and a thermostat, the samples were preserved better than in

Table 2

WEIGHT LOSS DURING DRYING OF THE TABLET MIXTURE AT DIFFERENT STAGES OF PRODUCTION

Tartaric acid isomer	**Weight loss during drying, %**				**Note**
	Tablet mixture	**Tablet mixture after moistening**	**Tablets after tableting**	**Tablets after drying**	
Racemate (D,L)	1.60	2.08	1.75	0.94	Some of the tablets were selected before drying and packed separately
D-isomer	1.47	2.08	1.59	0.80	

Table 3

TYPES OF MUKALTIN TABLETS PROVIDED FOR STORAGE

Medicine	Physical and chemical properties of tartaric acid	Weight loss during drying of tartaric acid, %	Primary packing materials
Reference substance			PVC
Mukaltin, 50 mg tablets	Racemate	1.75	PVC
			PVDC
		0.94	PVC
			PVDC
	D-isomer	1.59	PVC
			PVDC
		0.80	PVC
			PVDC

the climatic chamber, but also had an unsatisfactory appearance (the packaging was swollen).

When stored at room temperature, the best stability was shown by samples of tablets 7 and 8. The appearance of these samples did not change. Tablets produced by Obnovlenie JSC, repackaged in a PVC blister, darkened and swollen in all storage conditions, while in the original packaging, there was no change in appearance.

The studies provided allow us to make a number of findings.

- The residual moisture content of the finished tablet plays an important role in ensuring the stability of the mucaltin tablets. Mukaltin tablets that have passed the drying stage show higher stability.
- The tablet fractural strength after storage in the climatic chamber decreased by 3 times, when stored at room temperature, the strength practically did not change, and at 40°C increased by 1.5 times.

Table 4

EVALUATION OF THE APPEARANCE OF MUKALTIN TABLETS

Sample number	Appearance								Sum of values
	Discoloration				Swelling				
	cc	therm	At r.t.	package	cc	therm	At r.t.	package	
1	3	1	2	2	4	4	3	2	**21**
2	3	2	2	3	4	4	2	3	**23**
3	3	2	2	2	4	4	2	3	**22**
4	3	1	2	2	4	4	3	4	**23**
5	3	1	2	3	4	3	3	3	**22**
6	3	0	1	3	4	2	3	4	**20**
7	3	0	0	1	4	2	0	2	**12**
8	3	0	0	1	4	2	0	2	**12**
9	3	0	1	2	4	3	3	4	**20**

Table 5

GENERAL CHARACTERISTICS OF TABLETS AFTER 3 MONTHS OF TESTING

Sample number	Tartaric acid isomer	The presence of the drying stage	Type of a film	Appearance of tablets, total score	Average tablet weight, g	Fractural strength, N	Weight loss during drying, %
1	reference substance		PVC	**21**	0,254	33	3.93
2	D–L	–	PVC	**23**	0,236	28	3.80
3	D–L	–	PVDC	**22**	0,242	30	3.11
4	D	–	PVDC	**23**	0,217	26	7.01
5	D	–	PVC	**22**	0,254	31	3.93
6	D–L	+	PVC	**20**	0,233	28	7.15
7	D–L	+	PVDC	**12**	0,280	35	2.27
8	D	+	PVDC	**12**	0,291	38	2.04
9	D	+	PVC	**20**	0,262	33	3.74

- According to experience, PVDC packaging has satisfactory barrier parameters. Only in this package, the tablets were preserved for 3 months. The tablet original packaging made by Obnovlenie JSC turned out to be thinner than the one used in this experiment (150 μm versus 200 μm). Probably, this thickness allows the package to remain permeable to the products of the gas formation reaction, and, therefore, the resulting carbon dioxide can escape through the pores of the material without damaging the blister, thereby preserving the marketable appearance of the medicine.

Based on the conducted studies, it can be concluded that from the studied factors, the critical parameters of the production process of mukaltin tablets include the following:

1. Humidity of semi-products and finished product. To achieve the best stability of the medicine under the stated conditions, it is recommended to include the drying stage of the finished tablets into the production process.

2. Packaging material. Primary blister pack made of PVDC provides better stability of the medicine.

3. Storage conditions. Mukaltin tablets are best stored at temperature of up to 25°C and relative humidity 40±5%.

Factors that do not have a pronounced effect on the quality and stability of mukaltin tablets and are not related to critical parameters:

- moisture of the initial components;
- tartaric acid isomerization.

This study shows that various production factors can have a pronounced effect on the stability of tablets containing dry extracts. Failure to comply with these requirements may result in the reject of the entire batch. Therefore, when developing medicines of this group, it is necessary to carefully determine the critical parameters and strictly control them throughout the entire process.

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UDC 615.12
https://www.doi.org/10.34907/JPQAI.2021.59.94.008

PRODUCTION OF SUSPENSIONS IN PHARMACY: CONTRADICTIONS AND INCONSISTENCIES IN THE REGULATORY FRAMEWORK

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The method of comparative analysis was used to study the regulatory documents governing the compounded production of suspensions in Russian pharmacies and pharmacies in the neighboring countries and far-abroad countries. The official formulas were considered, the production of which is difficult in accordance with the existing regulatory requirements. The need to improve the regulatory framework governing the production of suspensions in pharmacies is shown.

Keywords: suspensions, pharmaceutical preparation, regulatory documents

In modern conditions, the compounded production of dosage forms in pharmacy conditions continues to be in demand. Prescriptions for preparation of medicines are written by physicians of various specialties, including dermatologists. One of the most popular dosage forms in dermatological practice is suspensions [1].

In dermatocosmetological practice, suspensions are often called "magma" or liquid powders. Water suspensions have a cooling, superficial vasoconstricting and drying effect, as well as an anti-inflammatory effect. Water "magmas" are a convenient substitute for dusting powders, since after evaporation and drying of the aqueous dispersion medium, powdery substances settle on the skin as a uniform thin layer and remain on it for a longer time. Unlike dusting powders, "magmas" do not dry the skin so much and act longer, have a cooling, superficial vasoconstricting and drying effect and are used to treat superficial acute inflammatory processes without oozing lesion and excessive dryness of the skin. The quantitative ratio of the dispersed phase and the dispersion medium varies depending on the purpose for which this dosage form is used: if an anti – inflammatory effect is required, then the amount of the dispersion medium is increased – with a pronounced inflammatory reaction of the skin, more liquid is added (40–50%). To provide an antiseptic effect and accelerate drying, ethyl alcohol (10–15%) is added. If the mixture is required not to be dried for a long time after application to the skin, as well as to enhance the emollient action, glycerin (5–10%) shall be added. If it is necessary to provide a covering effect, take equal amounts of powdered substances and liquid [2]. Thus, varying the composition of the suspension allows dermatologists to choose the most optimal composition based on the individual problems of a particular patient, which in principle cannot be done for commercially available drugs.

Consider suspensions from the perspective of the pharmacologically active substances and excipients forming this dosage form and technologies in order to assess the availability of

the technology and the feasibility of this dosage form.

According to the State Pharmacopoeia of the XIV edition, "suspensions are a liquid dosage form that is a heterogeneous dispersed system containing one or more solid active ingredients distributed in a liquid dispersion medium. The size of solid particles in suspensions can vary widely – from 0.1 to 10 μm or more" [3]. Hydrophilic substances used in dermatocosmetological practice include zinc oxide, magnesium oxide, basic magnesium carbonate, basic bismuth nitrate, white clay, starch. As a dispersion medium, purified water or water and water-alcohol solutions of medicinal substances are used. Suspensions of hydrophilic substances are prepared by the dispersion method without the addition of a stabilizer. For preparation of suspensions of hydrophobic substances (sulfur, menthol, thymol, sulfonamide preparations, etc.), it is necessary to add a stabilizer, which is taken either an equal amount in the case of preparation of a suspension of substances with pronounced hydrophobic properties, or half the amount in the case of preparation of suspensions of substances with non-pronounced hydrophobic properties.

As features of the technology, the State Pharmacopoeia of the XIV edition establishes the possibility of preparation of suspension by dispersing a solid internal phase containing an insoluble, pre-crushed active ingredient (ingredients) with a liquid dispersion medium or by other methods. As excipients in suspensions, buffer solutions, stabilizers, correctives, preservatives, antioxidants, dyes and other substances approved for medical application can be used. The introduction of preservatives, antioxidants and dyes into the suspension is most relevant for mass-produced medicines, and their absence in compounded suspensions can be considered as a positive characteristic, since the suspension composition is not unnecessarily "become heavier" due to excipients, which independently can cause deterioration in a number of patients with dermatological problems [4].

The production of dosage forms in pharmacy conditions is regulated by the Order of the Ministry of Health of the Russian Federation No. 751n of 2015 "On Approval of the rules for preparation and dispensation of medicines for medical use by pharmacy organizations, individual entrepreneurs who have a license for pharmaceutical activities" [5]. In this order, the technology of suspensions is considered together with the technology of emulsions, and the order establishes that (quote) "Suspensions (and emulsions) are prepared in a mortar or using mixers of various designs. Suspensions (and emulsions), regardless of concentration, are prepared by weight. When making suspensions (and emulsions) in mixers, all the ingredients are placed into the apparatus and mixed until a homogeneous mixture is obtained. The mixing time is specified by the properties of the medicines and the design of the apparatus. Suspensions are not subject to filtration. Preparation of suspensions in a mortar by grinding the powdery insoluble medicines is provided according to the rules for the production of powders, followed by dispersion with an optimal amount of liquid (in the amount of 1/2 of the mass of the crushed medicine or the crushed medicine and stabilizer) and dilution with a dispersion medium. Preparation of a suspension of hydrophobic medicinal products is carried out using stabilizers of heterogeneous systems specified in the Appendix to these Rules, and taking into account the physico-chemical properties of medicinal products and stabilizers, as well as the method of application of the dosage form".

Taking into account all of the above, when considering the technology of real dosage forms prescribed by dermatologists, serious difficulties arise. The issues of calculations in the technology of suspensions were not thought out in previous regulatory documents, namely in orders No. 412 of 1972 [6], No. 435 of 1990 [7], No. 308 of 1997 [8],

it was often difficult to follow their requirements, at the present moment, according to order No. 751n of 2015, it is simply impossible to do this for a number of prescriptions of suspensions.

According to the previously existing regulatory documentation for preparation of liquid dosage forms in pharmacies – order No. 435 of 1990, and then order No. 308 of 1997, there was a differentiation: suspensions in the concentration of medicinal substances up to 3% were prepared in weight-volume concentration, suspensions in the concentration of medicinal substances of 3% and above were prepared by weight. The orders contained specific official formulas, on the example of which the preparation of suspensions was considered. For example, the following dosage form:

Take:

Zinc oxide	
Talcum powder	by 20.0
Glycerin	30.0
Purified water	100.0

Mix. Give. Specify "For external use"

In the order, the pharmaceutics was described as follows: In a mortar, zinc oxide is mixed with talc, dispersed with glycerin (the latter is pre-weighed into a bottle), 50 ml of water is added by parts while stirring. With the remaining water, the suspension is washed off into a bottle for dispensing. Since the concentration of the suspension is more than 3%, then, according to the previously valid regulatory document, the preparation was carried out by weight, which is 170 g, the pharmaceutics is adequate. However, in the following example, given in the same regulatory document, nothing was clear in terms of determining the total mass and calculations.

Take:

Precipitated sulphur	7.0
Salicylic acid	2.0
Streptocide	3.0
Camphora	3.5
Glycerin	3.0
Ethyl alcohol,	50 ml
Boric acid solution	3% 50 ml

The order provided the following recommendations for preparation. In a mortar, streptocide and sulfur are crushed with alcohol and glycerin. The contents of the mortar are washed off with a solution of boric acid in a bottle for dispensing, where an alcoholic solution of salicylic acid and camphor is added in the remaining amount of 90% ethyl alcohol.

Here, the suspension is formed by sulfur, streptocide and camphor, their total content is more than 3% – respectively, the preparation was carried out by weight. However, there was no information about the total mass. It is difficult to determine the total weight of the dosage form, if the composition includes an aqueous solution of boric acid and 90% ethyl alcohol. If alcohol can still be converted to mass, taking into account the density, the value of which can be taken from the alcoholometric table No. 1 of the SP, then the densities of boric acid solutions of various concentrations (in recipes there can be 0.5%, 1%, 2% solutions) are not listed in the Appendix to the Order (both old and current) (only the densities of boric acid solutions of 3% and 4% concentrations are given).

Thus, it is impossible to determine the total mass of the dosage form accurately. Accordingly, it will be difficult to analyze its quality. The current Order of the Ministry of Health of the Russian Federation No. 751n of 2015, which establishes the preparation of suspensions by weight, does not consider any examples illustrating the calculations and production of dosage forms, so the interpretation of this document in terms of the technology of real suspensions may be different. On January 1, 2021, the Order of the Ministry of Health of the Russian Federation No. 308 of 1997, which clarified some issues of suspension technology, became invalid. Since various

suspensions are also widely prescribed by dermatologists at the present time, it is absolutely impossible to adequately interpret the calculations for determining the total mass of the abovementioned dosage forms in accordance with the current regulatory document.

Consider the following example of another prescription:

Take:
Basic Bismuth Nitrate 6.0
Sodium hydrocarbonate 4.0
Purified water 200.0
Mix. Dispense. Specify "Lotion"

If we consider the preparation of this dosage form according to Order No. 751n of 2015, it should be prepared by weight, so the total weight will be 210 grams. The technology itself is not difficult. But if the same prescription is prescribed by a doctor in a different form, namely:

Take:
Basic Bismuth Nitrate 6.0
Sodium hydrocarbonate solution 2% 200.0
Mix. Dispense. Specify "Lotion",

then, it becomes completely incomprehensible the total weight of the dosage form and the calculations during its preparation. So, when determining the total weight, it would be necessary to take into account the density of the sodium bicarbonate solution, which is not possible for each of its concentrations, which may be in the official formula. If you make calculations for the preparation of a sodium bicarbonate solution in a weight concentration (that is, when determining the volume of water required for preparation, subtract the amount of sodium bicarbonate from the amount of water), this will contradict the legal framework, since in the preparation of aqueous solutions the weight-volume method of production of dosage forms is assumed. If you make calculations for the preparation of a sodium bicarbonate solution in a weight concentration (that is, when determining the volume of water required for preparation, subtract the amount of sodium bicarbonate from the amount of water), this will contradict the legal framework, since the weight-volume method of production of dosage forms is adopted for the preparation of aqueous solutions. If we prepare a solution of sodium bicarbonate, which is part of the suspension, in a weight-volume concentration, according to Order No. 751n of 2015, then this will again contradict the requirements of the Order on the mass method of preparation of the suspension, since we must take 4.0 grams of sodium bicarbonate and 200 ml of purified water for its preparation (the increase in the volume of the solution in this case is not taken into account, since it is in compliance with the standard permissible deviations of the total volume of the solution), while the total mass of such a solution will be 204 grams, respectively, the weight of the suspension itself will be 210 grams. This is not consistent with the recipe and the requirements of Order No. 751n of 2015. If the prescriptions are multi-component, then the problem is even more pronounced.

Let's consider how the issue of suspension technology is solved in the near abroad countries.

Suspensions are still an actual dosage form in pharmacies in Belarus, primarily for the treatment of dermatological diseases [9]. The preparation is regulated by the pharmacopoeial monograph "Suspensions" of the State Pharmacopoeia of the Republic of Belarus. The basic rules set out in this monograph are similar to the rules set out in the Order of the Ministry of Health of the Russian Federation No. 308 of 1997 (if the content of substances is up to 3%, the suspension is prepared by mass-volume method, if 3% or more, it is prepared by weight), but along with this there are significant differences. Firstly, the regulatory framework does not prohibit the use of concentrated solutions

of pharmaceutical substance, and secondly, the use of ready-made monopreparations instead of active pharmaceutical ingredients is allowed. But – just as in the Russian Order – there are no clear recommendations on how to make calculations if the content of pharmaceutical substances exceeds 3%, and the prescription contains a solution of a pharmaceutical substance [10].

In Ukraine, the production of medicines in pharmacies is regulated by the State Pharmacopoeia of Ukraine and the Orders of the Ministry of Health, which also do not have clear recommendations on how to act in the situation that we are considering [11]. Just as in Belarus, regulatory documents do not prohibit the use of concentrated solutions in the preparation of suspensions, and in the preparation of suspensions for oral and external use, the use of ready-made medicines is allowed, if this is specified by the physician in the inscription.

In accordance with the order of the Ministry of Health of the Republic of Kazakhstan No. 142 of 15.12.2004, suspensions with the content of insoluble solid pharmaceutical substances of 3% or more are prepared by weight, the concentrated solutions of water-soluble pharmaceutical substances are not used in the preparation of suspensions. This provision fully corresponds to the requirements of the Order of the Ministry of Health of the Russian Federation No. 308 of 1997. Both Orders contain almost identical examples. There are no differences in the approaches to the preparation of suspensions. But when analysing the following prescription:

Take:	
Precipitated sulphur	7.0
Salicylic acid	2.0
Glycerin	5.0
Streptocide	3.0
Camphora	3.5
Ethyl alcohol	50.0
Boric acid solution 3%	50 ml

the authors of the regulatory document rationally approached the determination of the total mass (weight) of the dosage form: the weight of the suspension is 112.41 g, since the weight of 50 ml of 90% alcohol is 41.46 g (density – 0.829 g/ml); the weight of 37.5 ml of a 4% solution of boric acid is 37.95 g (density – 1.010 g/ml) [12].

Suspensions of compounded preparation remain a popular dosage form in pharmacies of far-abroad countries [13]. In the educational literature of the United States and Great Britain, the issues of suspension technology are discussed in detail. The main difference in the approach to their production is that all suspensions are prepared by volume. After crushing and mixing, the active ingredients are dispersed in a mortar with a small volume of the liquid phase (water or solutions of water-soluble substances), diluted with the liquid phase, transferred to a measuring vessel and brought to the desired volume with water [14,15]. The production of suspensions by this method does not provide for the need to take into account the densities of incoming solutions of various pharmaceutical substances, thus, there is no need to perform calculations to determine the total weight of the dosage form.

CONCLUSIONS

In compounded production, suspensions remain an actual and popular dosage form. Analysis of the regulatory framework of the post-Soviet states shows that their Orders were based on the Orders of the Ministry of Health of the USSR and the Russian Federation. Common approaches to the preparation of suspensions have been preserved, but the regulatory framework requires careful consideration, specification and refinement in order to adequately interpret the technology of suspensions, depending on the method of prescribing the official formula.

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ISSN 2309-6039