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# To the question of standardization of dry extract of oat fruits seeds (*Avena sativa* L.)

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**Abstract**
A dry aqueous extract was obtained by the method of maceration-circulation from the fruits of oats sowing. Taking into account the principle of "through standardization", indicators of quality and standardization of oats of dry sowing seed extract have been developed - the content of the sum of polysaccharides, loss in weight during drying, heavy metals content, microbiological purity. The conditions and shelf life of the extract were investigated.

**Keywords:** Heavy metals, extract, microbiological purity, oat, standardization

## Introduction
As is known, extracts are widely used in the practice of pharmacy as a separate dosage form and are included in other dosage forms. Dry extracts are concentrated drugs of solid consistency obtained from dried medicinal plant materials. Suitable extractants may be added to the dry extracts. The advantage of dry extracts is that it solves the problem of standardizing the quality of the feedstock and finished products. In ordinary aqueous-alcoholic extraction, the solvent is removed at a low temperature, therefore, beneficial substances are retained as much as possible. When extraction in vacuum at low temperatures, the maximum biological activity of the active substances is preserved and the high quality of medicinal substances is guaranteed, even when dry, they contain all biologically active substances inherent in this type of raw material, are convenient for storage and transportation. A high concentration of solids allows the use of ready-made extracts in small quantities. Also, the possibility of combining extracts at the manufacturing stage with other functional products is being numbered [1, 2].
Considering the above, as well as to expand the range of dosage forms from plant materials and simplify the application procedure based on the seeds of oats, a dry aqueous extract was obtained by maceration-circulation method. The yield of dry extract is 30±1.5 g.
The aim of this study is to develop methods for standardizing the dry extract of oat seeds.

## Materials and methods
The objects of the study were samples of aqueous extracts of fruits of oats sowing, obtained in a ratio of 1:10. The dry extract was obtained from dried fruits of sowing oats, harvested in the Samarkand region of the Republic of Uzbekistan during the full ripening of fruits in 2017-2018 (September-October). After collection, the raw materials were dried in air, under a canopy at a temperature of 15-20 ºC.
The samples were determined by D.Z. Berdibaeva, a researcher at the Botanical Garden. The climate of the territory of the Samarkand region can be divided into two zones. The northern part and the extreme west of the region belong to the continental climate, and the remaining part (center, south and east) of the region covers the subtropical intracontinental climate. Both climates represent hot and dry summers with partly cold winters. The average annual temperature is +16.5 °C; the average January temperature is 0.2 °C, the average July temperature is +27.0 °C. The absolute minimum temperature was - 26 °C, the absolute temperature maximum +58 °C. On average, 310-330 mm of precipitation falls on the territory of the region per year (the main part of the precipitation falls in spring and autumn). The growing season lasts 218-220 days.
Dried fruits (caryopsis) are large, full, almost cylindrical or pear-shaped, greenish-yellow in color. The length of the fruit is 8-12 mm, the width is 0.2-0.3 mm. The caryopsis is filmy (densely covered by floral films, but not fused with them) or bare (freely lying between flower scales), covered over the entire surface with pressed hairs, 8-11 mm long, has a pronounced

longitudinal groove. A caryopsis with flowering scales does not grow together and consists of a shell, an endosperm and an embryo. The outer part of the membrane is formed from the walls of the ovary; it is the fruit membrane. Under the fruit membrane is the seed membrane, which is formed from two membranes of the ovule. The embryo consists of a scutellum, primary roots in the form of small tubercles and a kidney. The scutellum is located between the embryo and the endosperm and is the only cotyledon of the caryopsis. The kidney consists of a rudimentary stem ending in a cap of germinal leaves. The embryo occupies a small part of the seed. The bulk of the grain is the endosperm. The peripheral layer of the endosperm is called the aleuron layer and is located under the seminal membrane. The aleurone layer is rich in nutrients, and endosperm-starch. Flavourless, with original taste.

The dry extract of oat seeds was standardized according to the requirements of the SP XI "Extracts" article [3] according to the following indicators: description, authenticity, weight loss upon drying, heavy metal content, quantitative determination of the content of active substances (sum of polysaccharides) and microbiological purity.

External signs of the dry extract were determined with the naked eye. Color in daylight, smell - when rubbed.

*Description.* The dry extract is a loose powder of brown color with a characteristic odor, homogeneous and hygroscopic. It is soluble in water and diluted ethyl alcohol, insoluble in organic solvents.

An analysis of the literature shows that the fruits of oats are a valuable source of biologically active substances. The dominant and determining pharmacological effect of this species are polysaccharides. In this connection, the chemical standardization of the dry extract was carried out precisely for this group of natural compounds.

*Authenticity.* Authentication was carried out by qualitative reactions to active substances - polysaccharides [4].

Methodology. 10 g of dry extract was placed in a flask with a capacity of 250 ml, 200 ml of water was added, the flask was attached to a reflux condenser and boiled with stirring on an electric stove for 30 minutes. 30 ml of 95% alcohol were added to 10 ml of the resulting solution and mixed, the appearance of flocculent clots precipitating upon standing indicated the presence of polysaccharides.

*Mass loss on drying.* The loss in mass when dried in a dry extract of oat seeds was determined according to SP XI: about 0.5 g of dry extract (accurately weighed) was dried in an oven at 102.5 + 2.5 °C for five hours, then cooled and weighed. The loss in mass upon drying was 3.3 - 4.2%.

*Quantitative determination of active substances (amount of polysaccharides).* About 10 g of dry extract was placed in a flask with a capacity of 250 ml, 200 ml of water was added, the flask was attached to a reflux condenser and boiled with stirring on an electric stove for 30 minutes. The extraction with water was repeated 2 more times, using the first time 200 ml, the second time 100 ml water. The aqueous extracts were combined and centrifuged at 5000 rpm for 10 minutes and decanted into a 500 ml volumetric flask through 5 layers of gauze, inserted into a 55 mm diameter glass funnel and pre-moistened with water. The filter was washed with water and the volume of the solution was adjusted to the mark with water (solution A).

25 ml of solution A was placed in a centrifuge tube, 75 ml of 95% alcohol were added, mixed, heated in a water bath to 300 °C for 5 min. After one hour, the contents were centrifuged at a speed of 5000 rpm for 30 minutes. The supernatant was filtered under vacuum at a residual pressure of 13-16 kPa through a 40 mm diameter POR 16 glass filter dried to constant weight at a temperature of 100-105 °C. The precipitate was quantitatively transferred to a filter and washed successively with 15 ml of a solution of 95% alcohol in water (3:1), 10 ml of acetone, 10 ml of ethyl acetate. The filter sediment was dried first in air, then at a temperature of 100-105ºC to constant weight. The content of polysaccharides in terms of absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{(m_2 - m_1) \times 500 \times 100 \times 100}{m \times 25 \times (100 - W)}$$

where, $m_1$ is the filter mass in grams;
$m_2$ - mass of filter with sediment in grams;
m is the mass of raw materials in grams;
W is the loss in mass upon drying of the raw material as a percentage.

The results of quantification are presented in table 1.

**Table 1:** Metrological description of the method for the quantitative determination of polysaccharides in the dry extract of oats

| *f* | *x* | $\bar{x}$ | $S^2$ | *S* | *t(pt)* | *Δx* | *Δ$\bar{x}$* | $E_1$% | *E*% |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 22.4<br>22.3<br>22.4<br>22.6<br>22.5 | 22.4 | 0.0525 | 0.2236 | 2.78 | 0.6216 | 0.2787 | 2.77 | 1.24 |

Note: N is the number of samples, f is the number of degrees of freedom, X is the arithmetic mean, S2 is the dispersion, S is the standard deviation, P is the significance level, t (pt) is the Student t-test, ΔX is the absolute error of the analysis, E is probable deviation.

## Results

As the results of the study showed, the content of polysaccharides in the studied dry extract ranges from 22.3-22.6%. Based on the data obtained, the norm of the content of polysaccharides in the dry extract of sowing oats has been established at least 20%.

*The content of heavy metals.* 1 ml of concentrated sulfuric acid was added to 1 g of dry extract, carefully burned, calcined. The obtained residue was treated with heating 5 ml of a saturated solution of ammonium acetate, filtered through an anhydrous filter, washed with 5 ml of water, and the filtrate volume was adjusted to 200 ml. 10 ml of the resulting solution were subjected to measurement of the content of heavy metals (the proposed level is not more than 0.01% by weight).

Determination of the content in the dry extract of toxic heavy metals - lead and cadmium, which the Joint FAO and WHO Codex Alimentaries Commission refers to are among the components that are subject to priority control in the international food trade. The quantitative content of lead and cadmium was carried out by an inductively coupled plasma optical emission spectrometric method (Optima-2400 DV device, Perkin Elmer, USA) [5, 6].

The experimental results are shown in table 2.

**Table 2:** The content of heavy metals in the dry extract of oats

| Item to be defined | Maximum allowable concentrations in food, µg/g * | Actual content, mcg/g |
|---|---|---|
| Pb | 6.0 | 0.0 |
| Cd | 1.0 | 0.0 |

Note: * - the limit content of heavy metals in accordance with the requirements of the SP RF GPA.1.5.3.0009.15 [7].

As can be seen from the above data, the content of toxic heavy metals that are subject to priority control in the dry extract does not exceed permissible concentrations.

Next, the microbiological purity of the dry extract was determined in accordance with the requirements of SP XI [3, 8] and the amendment to the SP XI article "Methods of microbiological control of medicines" dated September 29, 2005, category 4A.

As a result of the study of five series of dry extract in 1 g of the drug, an average of $10^6$ aerobic bacteria, $10^3$ east and mold fungi (total), $10^2$ enterobacteria and other gram-negative bacteria were found. The bacteria Esherichia coli and Salmonella, Pseudomonas aeruginosa and Staphylococcus aureus were not detected.

The shelf life of the dry extract of oat seeds according to OST 42-2-70 *in vivo* was studied and it was found that it is more than 2 years.

**Discussion.**

As a result of the research, norms that regulate the authenticity and quality of the dry extract were determined (table. 3).

**Table 3:** Description of dry oats extract

| The name of indicators | Norm for dry extract |
|---|---|
| Description | Loose powder of brown color with a characteristic odor, homogeneous and hygroscopic. |
| Solubility | Soluble in water, dilute alcohol and insoluble in organic solvents. |
| Authenticity | Reaction with 95% alcohol, flocculent clots appear that precipitate upon standing (polysaccharides). |
| Mass loss on drying | Not more than 5% |
| Polysaccharides content | Not less than 20% |
| Heavy metals content | Not more than 0.01% |

**Conclusion**

Taking into account the principle of "through standardization", quality and standardization indicators of dry sowing oats dry extract have been developed - the content of the amount of polysaccharides (not less than 20%), the mass loss upon drying (not more than 5%), the content of heavy metals (not more than 0.01%), microbiological purity. The conditions and shelf life of the extract were investigated. The recommended shelf life of 2 years at a temperature of no higher than 20 °C and humidity in the room 60 - 65%.

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